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(71) Applicant: ZYMOGENETICS, INC. [US/US]; 4225 Roosevelt Way NE, Seattle, WA 98105 (US).

(72) Inventor: IRANI, Meher, H.; 3503 NE 100th, Seattle, WA 98125 (US).

(74) Agent: PARKER, Gary, E.; ZymoGenetics, Inc., 4225 Roosevelt Way NE, Seattle, WA 98105 (US).

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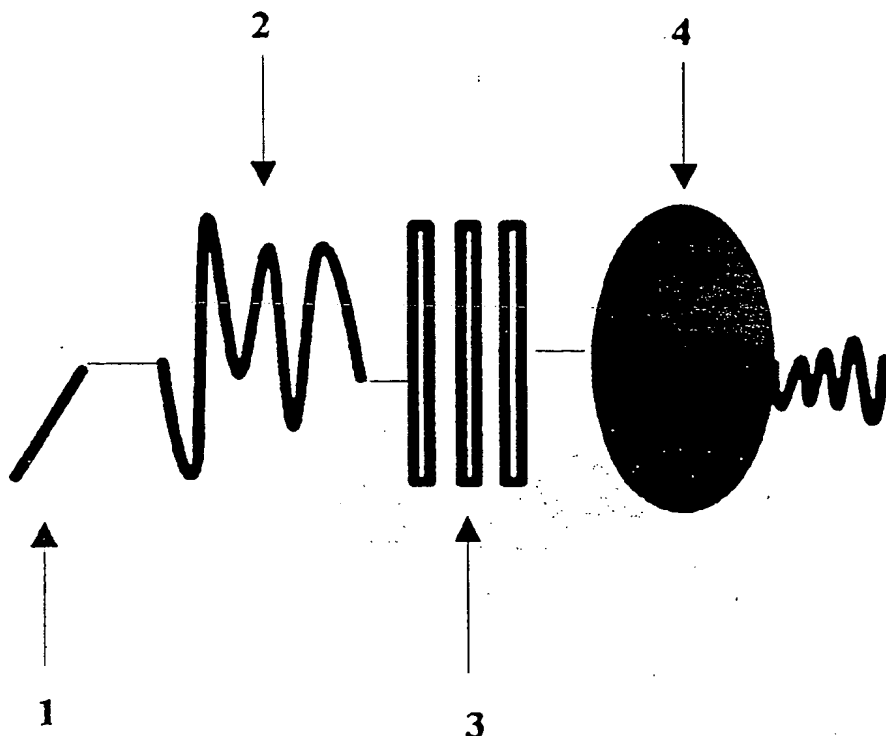
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(54) Title: HYBRID PROTEINS HAVING CROSS-LINKING AND TISSUE-BINDING ACTIVITIES

(57) Abstract

Hybrid proteins having cross-linking and tissue-binding activities, DNA molecules encoding such proteins and methods for producing the hybrid proteins from recombinant host cells are disclosed. The hybrid proteins disclosed herein are useful in tissue sealant and wound healing formulations.



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DescriptionHybrid Proteins Having Cross-Linking and Tissue-Binding
Activities5 Technical Field

 The present invention relates generally toward
methods for producing recombinant hybrid proteins, and
more specifically, to methods for producing hybrid
proteins from host cells through the use of recombinant
10 DNA techniques.

Background of the Invention

 The utilization of tissue sealants to replace or
augment the use of mechanical wound closure devices has
15 expanded in recent years in many surgical and trauma
applications. Tissue sealants include biological
adhesives (e.g. fibrin-based adhesives) and synthetic
preparations (e.g. cyanoacrylates). It is widely
acknowledged that the use of synthetic preparations of
20 tissue sealants is limited due to their toxicity and
limited applications. Biological tissue adhesives have
demonstrated utility in cases where the use of mechanical
devices to close wounds is insufficient, such as in
joining blood vessels, closing holes in the dura, and in
25 surgery on small or delicate tissues such as in the eye or
ear.

 Fibrin-based biological tissue adhesives
generally contain fibrinogen, factor XIII and thrombin as
principal ingredients, although in practice biological
30 tissue adhesives are derived from whole blood and contain
additional blood proteins. The fibrinogen and factor XIII
components of these adhesives are prepared from pooled
human plasma by cryoprecipitation (e.g. U.S. Patents No.
4,377,572; 4,362,567; 4,909,251), by ethanol precipitation
35 (e.g. U.S. Patent No. 4,442,655) or from single donor
plasma (e.g. U.S. Patent No. 4,627,879; Spotnitz et al.,
Am. Surg. 55: 166-168, 1989). The resultant

fibrinogen/factor XIII preparation is mixed with bovine thrombin immediately before use to convert the fibrinogen to fibrin and activate the factor XIII, thus initiating coagulation of the adhesive.

5 Fibrin-based tissue adhesives, in their current form, have significant drawbacks that include poor standardization, lack of quality control from batch to batch and the possibility of transmission of human immunodeficiency virus (HIV), hepatitis virus and other
10 etiologic agents. While recombinant production of thrombin and factor XIII have been reported, and while these proteins might be used in biological tissue adhesives, the biological tissue adhesives still rely on large amounts of fibrinogen that is obtained from pooled
15 human blood. At present, current fibrin(ogen)-based tissue adhesives are not approved for use in the United States.

There is therefore a need in the art for tissue adhesive components, particularly components that
20 facilitate cross-linking to improve clot strength, that are prepared at high levels with reproducible activity levels and which do not carry the possibility of transmission of viral or other etiologic agents. The present invention addresses these needs by providing
25 recombinant hybrid proteins that provide cross-linking and tissue-adhesive properties and that may be prepared at high levels.

Disclosure of the Invention

30 Briefly stated, the present invention provides hybrid proteins having cross-linking and tissue-binding activities, DNA molecules encoding such hybrid proteins and methods for producing hybrid proteins by recombinant means. In one aspect, In one aspect of the invention, the
35 hybrid proteins comprise a tissue-binding domain from a first protein covalently linked to a cross-linking domain from a second protein. Within a related aspect of the

invention, the tissue-binding domain of the first protein is a heparin binding domain of thrombospondin, a heparin binding domain of fibronectin, a collagen binding domain of fibronectin or a cell binding domain of fibronectin.

5 Within a preferred embodiment, the tissue-binding domain of the first protein comprises the amino acid sequence of Sequence ID No. 6 from Alanine, amino acid 2 to Glutamic acid, amino acid number 926. Within another related

10 aspect of the invention, the cross-linking domain of the second protein comprises the carboxy-terminal 103 amino acids of loricrin, the ten amino acid repeat beginning with glutamine amino acid number 496 of involucrin or the 400 amino-terminal amino acids of the fibrinogen α chain.

15 Within a preferred embodiment of the invention, the tissue-binding domain of the second protein comprises the amino acid sequence of Sequence ID No. 6 from Glycine, amino acid number 928 to Proline, amino acid number 1336. Within a particularly preferred embodiment, the hybrid protein comprises the amino acid sequence of Sequence ID
20 No. 6 from alanine, amino acid number 2 to proline, amino acid number 1336.

The present invention provides DNA molecules encoding hybrid proteins of the present invention comprising a first DNA segment encoding a tissue-binding
25 domain from a first protein joined to a second DNA segment encoding a cross-linking domain from a second protein. In one embodiment, the first DNA segment comprises the nucleotide sequence of Sequence ID No. 5 from nucleotide 3 to nucleotide 2780. In another embodiment, the second DNA
30 segment comprises the nucleotide sequence of Sequence ID No. 5 from nucleotide 2784 to nucleotide 4013. In a preferred embodiment, the DNA molecule comprises the nucleotide sequence of Sequence ID Number 5 from nucleotide 3 to nucleotide 4013.

35 In related embodiments of the invention, DNA constructs are provided which comprise a DNA molecule encoding a hybrid protein, wherein said DNA molecule

comprises a first DNA segment encoding a tissue-binding domain from a first protein joined to a second DNA segment encoding a cross-linking domain from a second protein and wherein said DNA molecule is operably linked to other DNA segments required for the expression of the DNA molecule. Other embodiments of the invention concern host cells containing the DNA constructs of the present invention and methods of producing hybrid proteins.

10 Brief Description of the Drawings

Figure 1 discloses a representative hybrid protein containing (1) an N-terminal end-to-end inter-chain cross-linking domain, (2) a domain that promotes inter-chain cross-linking; (3) a domain that confers tissue binding activity; and (4) a carboxy-terminal domain that promotes end-to-end inter-chain cross-linking.

Figures 2-5 disclose absorbance time courses of representative cross-linking assays carried out in the presence of varying levels of factor XIII (activated to factor XIIIa via thrombin during the assay) or factor XIIIa.

Detailed Description of the Invention

The present invention provides novel hybrid proteins having cross-linking and tissue adhesive activities. The hybrid proteins comprise a cross-linking domain from a first protein covalently linked to a tissue-binding domain from a second protein. The hybrid proteins of the present invention are capable of cross-linking to themselves and to other proteins such as fibrin and fibrinogen and are capable of adhering to cell surfaces and/or extracellular matrix components. While not wishing to be bound by a graphical representation, Figure 1 shows a representative hybrid protein containing an N-terminal end-to-end inter-chain cross-linking domain; a domain that promotes inter-chain cross-linking; a domain that confers tissue binding activity; and a carboxy-terminal domain

that promotes end-to-end inter-chain cross-linking. As used herein, cross-linking refers to the formation of covalent bonds between polypeptides.

5 The hybrid proteins of the present invention are useful as components of tissue sealant formulations to provide matrix material and to improve clot strength over a wound site, and as components in formulations that promote wound healing. The proteins of the present invention may contain native (i.e. wild-type) protein
10 domains as well as domains that are allelic variants and genetically engineered or synthetic variants of the respective naturally occurring domains. Such variants are characterized by the presence of conservative amino acid substitutions and/or other minor additions, substitutions
15 or deletions of amino acids.

As used within the context of the present invention, tissue-binding domains include protein domains containing amino acid sequences that facilitate adherence to cell surfaces and/or to extracellular matrix components
20 such as collagen, fibronectin, hyaluronic acid and glycosaminoglycans. Fibronectin, for example, contains the sequence Gly-Arg-Gly-Asp-Ser (from amino acid 1614 through amino acid 1618 of Sequence I.D. No. 3) that has been shown to be central to cell recognition by the
25 fibronectin receptor (for review see Yamada, Current Opinion in Cell Biology 1: 956-963, 1989). The heparin binding domains of fibronectin (Sekiguchi et al., Proc. Natl. Acad. Sci. USA 77: 2661-2665, 1980), and thrombospondin (Zardi et al., EMBO J. 6: 2337-3342, 1987
30 and Gutman and Kornblihtt, Proc. Natl. Acad. Sci. USA 84: 7179-7182, 1987) contain sequences that recognize heparin sulfate-containing glycosaminoglycans which are extracellular matrix components. The collagen binding domain of fibronectin (Sekiguchi et al. ibid., 1980)
35 contains amino acid sequences that bind to the extracellular matrix component collagen.

Particularly preferred tissue-binding domains are the heparin binding domain of fibronectin, comprising the sequence of amino acids of Sequence I.D. No. 2 from alanine, amino acid number 1812 to valine, amino acid number 2171; the collagen binding domain of fibronectin, comprising the sequence of amino acids of Sequence I.D. No. 2 from glycine, amino acid number 282 to serine, amino acid number 608; and the amino terminal 229 amino acids of thrombospondin. In this regard, a particularly preferred tissue-binding domain is the cell-binding domain of fibronectin, comprising the sequence of amino acids of Sequence I.D. No. 3 from alanine, amino acid number 1357 to glutamic acid, amino acid number 1903. It will be evident to one skilled in the art that smaller portions of the cell-binding domain of fibronectin may be used within the hybrid proteins of the present invention, more particularly the sequence of amino acids of Sequence I.D. No. 3 from isoleucine, number 1532 through threonine, amino acid number 1631. As noted above, it is generally accepted that the sequence Gly-Arg-Gly-Asp-Ser (Amino acids 1614 to 1618 of Sequence I.D. No. 3) is central to cell recognition by fibronectin.

Cross-linking domains suitable for use in the hybrid proteins of the present invention are protein domains which contain amino acid sequences required for the formation of specific covalent bonds between peptide chains. In a preferred embodiment the inter-chain cross-links are covalent bonds formed by the action of a transglutaminase such as factor XIII, tissue transglutaminase, prostate transglutaminase, keratinocyte transglutaminase, epidermal transglutaminase or placental transglutaminase. Transglutaminases catalyze the formation of ϵ -(γ -glutamyl)lysine bonds between specific glutamine and lysine residues. However, other inter-chain cross-links, such as those formed by disulfide bonds, are also suitable cross-links. Suitable cross-linking domains include domains from the fibrinogen α chain, the

glutamine/lysine rich domains of loricrin that are involved in isodipeptide cross-link formation (Hohl et al., J. Biol. Chem. 266: 6626-6636, 1991), and at least one of the 10 amino acid-long repeats of involucrin (Cell 5 46: 583-589, 1986 and Etoh et al., Biochem. Biophys. Res. Comm. 136: 51-56, 1986). Preferred cross-linking domains are the carboxy-terminal 103 amino acids of loricrin (Hohl et al., ibid.) and the ten-amino acid repeat beginning with glutamine, amino acid number 496 of involucrin (Simon 10 et al. (J. Biol. Chem. 263: 18093-18098, 1988). A particularly preferred cross-linking domain comprises the 400 amino-terminal amino acids of the fibrinogen α chain (Doolittle et al., Nature 280: 464-468, 1979; Rixon et al., Biochemistry 22: 3250-3256, 1983). More 15 particularly, the amino acid sequence of Sequence ID No. 6 from Glycine, amino acid number 928 to Proline, amino acid number 1336 is preferred.

Although the hybrid proteins of the present invention may consist essentially of covalently linked 20 cross-linking and tissue binding domains, they may further contain domains that facilitate end-to-end covalent cross-linking. The γ chain of fibrinogen contains a domain that facilitates end-to-end cross-linking to another γ chain via ϵ -(γ -glutamyl)lysine bonds. This domain includes at 25 least the 19 carboxy-terminal amino acids and more preferably includes the amino-terminal 275 amino acids of the fibrinogen γ chain. The α chain of fibrinogen contains an amino-terminal domain that is involved in interchain disulfide bond formation between α chains. This domain 30 includes the amino-terminal portion of the α chain of fibrinogen from glycine, amino acid 36 to glycine, amino acid 67 of Sequence ID Number 4.

As will be evident to one skilled in the art, the hybrid proteins of the present invention may contain 35 domains of human and other animal proteins. Proteins containing domains suitable for use in the present invention from human and other animals and the DNA

molecules encoding such proteins have been reported. Involucrin, loricrin, fibrinogen and fibronectin, for example, have been studied in a variety of animals. DNA sequences encoding primate, canine and porcine involucrin have been reported (Djian and Green, Mol. Biol. Evol. 9: 417-432, 1992; Djian and Green, Proc. Natl. Acad. Sci. USA 88: 5321-5325, 1991 and Tseng and Green, Mol. Biol. Evol. 7: 293-302, 1990). Mehrel et al. (Cell 61: 1103-1112, 1990) have reported a DNA sequence encoding mouse loricrin. DNA sequences encoding rat and frog fibrinogen gamma chain have been reported (Haidaris and Courtney, Blood 79: 1218-1224, 1992 and Bhattacharya et al., Mol. Cell. Endocrinol. 72: 213-220, 1990; respectively). DNA sequences encoding chicken and lamprey fibrinogen α chains have been reported by Weissbach and Greininger (Proc. Natl. Acad. Sci. USA 87: 5198-5202, 1990) and Pan and Doolittle (Proc. Natl. Acad. Sci. USA 89: 2066-2070, 1992), respectively. DNA sequences encoding bovine and rat fibronectin have been reported by Petersen et al. (Proc. Natl. Acad. Sci. USA 80: 137-141, 1983) and Schwarzbauer et al., (Cell 35: 421-431, 1983). In general, it is preferred to prepare proteins that contain component domains from a single species to minimize the possibility of immunogenicity. Thus, the present invention provides hybrid proteins that can be used in human and veterinary medicine.

According to the present invention hybrid proteins having cross-linking and tissue adhesive activities are produced recombinantly from host cells transformed with a DNA construct comprising a DNA segment encoding a cross-linking domain from a first protein joined to a DNA segment encoding a tissue-binding domain from a second protein. As used within the context of the present invention, two or more DNA coding sequences are said to be joined when, as a result of in-frame fusions between the DNA coding sequences or as a result of the removal of intervening sequences by normal cellular

processing, the DNA coding sequences can be translated into a polypeptide fusion. Unless otherwise noted, the DNA segments may be joined in any order to result in a DNA coding sequence that can be translated into a polypeptide chain. Thus, the DNA segment encoding the tissue-binding domain may be joined to the 5' or the 3' end of the DNA segment encoding the cross-linking domain. However, as will be evident to one skilled in the art, the production of hybrid proteins that additionally include domains that facilitate end-to-end cross-linking will require that the DNA segments encoding such domains be positioned at the 5' and 3' termini of the molecules.

Thus the present invention also provides isolated DNA molecules encoding hybrid proteins comprising a cross-linking domain from a first protein covalently linked to a tissue-binding domain from a second protein. In general, cDNA sequences are preferred for carrying out the present invention due to their lack of intervening sequences which can lead to aberrant RNA processing and reduced expression levels. DNA molecules encoding human fibronectin (Dufour et al., Exper. Cell Res. 193: 331-338, 1991) and a human fibrinogen α chain (Rixon et al., Biochemistry 22: 3250-3256, 1983) may be obtained from libraries prepared from liver cells according to standard laboratory procedures. It will be understood however, that suitable DNA sequences can also be obtained from genomic clones or can be synthesized de novo according to conventional procedures. If partial clones are obtained, it is necessary to join them in proper reading frame to produce a full length clone, using such techniques as endonuclease cleavage, ligation, and loop-out mutagenesis.

DNA sequences encoding hybrid proteins of the present invention may be prepared from cloned DNAs using conventional procedures of endonuclease cleavage, exonuclease digestion, ligation and in vitro mutagenesis. Alternatively, DNA sequences encoding the cross-linking

and tissue-binding domains, such as those mentioned above, may be synthesized using standard laboratory techniques.

5 An exemplary DNA molecule encoding a hybrid protein having cross-linking and tissue-binding activities may be prepared by joining a DNA segment encoding at least the cell-binding domain of fibronectin and a DNA segment encoding at least an inter-chain cross-linking domain of fibrinogen at a convenient restriction site using synthetic adapters to facilitate in-frame joining of the
10 DNA segments. Alternatively, such DNA segments encoding hybrid proteins of the present invention may be prepared by joining the two domains at a convenient restriction site followed by loop-out mutagenesis to precisely remove unnecessary sequences and directly join the DNA segment
15 encoding the cell-binding domain of fibronectin with the DNA segment encoding the cross-linking domain of fibrinogen.

DNA segments encoding the hybrid proteins of the instant invention are inserted into DNA constructs. As
20 used within the context of the present invention, a DNA construct is understood to refer to a DNA molecule, or a clone of such a molecule, either single- or double-stranded, which has been modified through human intervention to contain segments of DNA combined and
25 juxtaposed in a manner that would not otherwise exist in nature. DNA constructs of the present invention comprise a first DNA segment encoding a hybrid protein operably linked to additional DNA segments required for the expression of the first DNA segment. Within the context
30 of the present invention, additional DNA segments will generally include promoters and transcription terminators, and may further include enhancers and other elements.

DNA constructs may also contain DNA segments necessary to direct the secretion of a polypeptide or
35 protein of interest. Such DNA segments may include at least one secretory signal sequence. Secretory signal sequences, also called leader sequences, prepro sequences

and/or pre sequences, are amino acid sequences that act to direct the secretion of mature polypeptides or proteins from a cell. Such sequences are characterized by a core of hydrophobic amino acids and are typically (but not
5 exclusively) found at the amino termini of newly synthesized proteins. DNA segments encoding secretory signal sequences are placed in-frame and in the correct spatial relationship to the DNA segment encoding the protein of interest in order to direct the secretion of
10 the protein. Very often the secretory peptide is cleaved from the mature protein during secretion. Such secretory peptides contain processing sites that allow cleavage of the secretory peptides from the mature proteins as they pass through the secretory pathway. A preferred
15 processing site is a dibasic cleavage site, such as that recognized by the Saccharomyces cerevisiae KEX2 gene. A particularly preferred processing site is a Lys-Arg processing site. Processing sites may be encoded within the secretory peptide or may be added to the peptide by,
20 for example, in vitro mutagenesis.

Preferred secretory signals include the α factor signal sequence (pre-pro sequence: Kurjan and Herskowitz, Cell 30: 933-943, 1982; Kurjan et al., U.S. Patent No. 4,546,082; Brake, U.S. Patent No. 4,870,008), the PHO5
25 signal sequence (Beck et al., WO 86/00637), the BAR1 secretory signal sequence (MacKay et al., U.S. Patent No. 4,613,572; MacKay, WO 87/002670), the SUC2 signal sequence (Carlsen et al., Molecular and Cellular Biology 3: 439-447, 1983). Alternately, a secretory signal sequence may
30 be synthesized according to the rules established, for example, by von Heinje (European Journal of Biochemistry 133: 17-21, 1983; Journal of Molecular Biology 184: 99-105, 1985; Nucleic Acids Research 14: 4683-4690, 1986).

Secretory signal sequences may be used singly or
35 may be combined. For example, a DNA segment encoding a first secretory signal sequence may be used in combination with a DNA segment encoding the third domain of barrier

(described in U.S. Patent No. 5,037,243, which is incorporated by reference herein in its entirety). The DNA segment encoding the third domain of barrier may be positioned in proper reading frame 3' of the DNA segment of interest or 5' to the DNA segment and in proper reading frame with both the DNA segment encoding the secretory signal sequence and the DNA segment of interest.

The choice of suitable promoters, terminators and secretory signals is well within the level of ordinary skill in the art. Methods for expressing cloned genes in Saccharomyces cerevisiae are generally known in the art (see, "Gene Expression Technology," Methods in Enzymology, Vol. 185, Goeddel (ed.), Academic Press, San Diego, CA, 1990 and "Guide to Yeast Genetics and Molecular Biology," Methods in Enzymology, Guthrie and Fink (eds.), Academic Press, San Diego, CA, 1991; which are incorporated herein by reference). Transformation systems for other yeasts, including Hansenula polymorpha, Schizosaccharomyces pombe, Kluyveromyces lactis, Kluyveromyces fragilis, Ustilago maydis, Pichia pastoris, Pichia guilliermondii and Candida maltosa are known in the art. See, for example, Gleeson et al., J. Gen. Microbiol. 132:3459-3465, 1986 and Cregg, U.S. Patent No. 4,882,279.

Proteins of the present invention can also be expressed in filamentous fungi, for example, strains of the fungi Aspergillus (McKnight et al., U.S. Patent No. 4,935,349, which is incorporated herein by reference). Methods for transforming Acremonium chrysogenum are disclosed by Sumino et al., U.S. Patent No. 5,162,228, which is incorporated herein by reference.

Other higher eukaryotic cells may also be used as hosts, including insect cells, plant cells and avian cells. Transformation of insect cells and production of foreign proteins therein is disclosed by Guarino et al., U.S. Patent No. 5,162,222 and Bang et al., U.S. Patent No. 4,775,624, which are incorporated herein by reference. The use of Agrobacterium rhizogenes as a vector for

expressing genes in plant cells has been reviewed by Sinkar et al., J. Biosci. (Bangalore) 11:47-58, 1987.

Expression of cloned genes in cultured mammalian cells and in E. coli, for example, is discussed in detail in Sambrook et al. (Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, NY, 1989; which is incorporated herein by reference). In addition to E. coli, Bacillus and other genera are useful prokaryotic hosts for expressing foreign proteins. As would be evident to one skilled in the art, one could express the proteins of the instant invention in other host cells such as avian, insect and plant cells using regulatory sequences, vectors and methods well established in the literature.

In yeast, suitable vectors for use in the present invention include YRp7 (Struhl et al., Proc. Natl. Acad. Sci. USA 76: 1035-1039, 1978), YEpl3 (Broach et al., Gene 8: 121-133, 1979), POT vectors (Kawasaki et al, U.S. Patent No. 4,931,373, which is incorporated by reference herein), pJDB249 and pJDB219 (Beggs, Nature 275:104-108, 1978) and derivatives thereof. Preferred promoters for use in yeast include promoters from yeast glycolytic genes (Hitzeman et al., J. Biol. Chem. 255: 12073-12080, 1980; Alber and Kawasaki, J. Mol. Appl. Genet. 1: 419-434, 1982; Kawasaki, U.S. Patent No. 4,599,311) or alcohol dehydrogenase genes (Young et al., in Genetic Engineering of Microorganisms for Chemicals, Hollaender et al., (eds.), p. 355, Plenum, New York, 1982; Ammerer, Meth. Enzymol. 101: 192-201, 1983). In this regard, particularly preferred promoters are the TPI1 promoter (Kawasaki, U.S. Patent No. 4,599,311, 1986) and the ADH2-4^C promoter (Russell et al., Nature 304: 652-654, 1983; Irani and Kilgore, U.S. Patent Application Serial No. 07/631,763, CA 1,304,020 and EP 284 044, which are incorporated herein by reference). The expression units may also include a transcriptional terminator. A

preferred transcriptional terminator is the TPI1 terminator (Alber and Kawasaki, *ibid.*).

Host cells containing DNA constructs of the present invention are then cultured to produce the hybrid proteins. The cells are cultured according to standard methods in a culture medium containing nutrients required for growth of the particular host cells. A variety of suitable media are known in the art and generally include a carbon source, a nitrogen source, essential amino acids, vitamins, minerals and growth factors. The growth medium will generally select for cells containing the DNA construct by, for example, drug selection or deficiency in an essential nutrient which is complemented by a selectable marker on the DNA construct or co-transfected with the DNA construct.

Selection of a medium appropriate for the particular host cell used is within the level of ordinary skill in the art. Yeast cells, for example, are preferably cultured in a chemically defined medium, comprising a non-amino acid nitrogen source, inorganic salts, vitamins and essential amino acid supplements. The pH of the medium is preferably maintained at a pH greater than 2 and less than 8, preferably at pH 6.5. Methods for maintaining a stable pH include buffering and constant pH control, preferably through the addition of sodium hydroxide or ammonium hydroxide. Preferred buffering agents include succinic acid and Bis-Tris (Sigma Chemical Co., St. Louis, MO). Yeast cells having a defect in a gene required for asparagine-linked glycosylation are preferably grown in a medium containing an osmotic stabilizer. A preferred osmotic stabilizer is sorbitol supplemented into the medium at a concentration between 0.1 M and 1.5 M, preferably at 0.5 M or 1.0 M. Cultured mammalian cells are generally cultured in commercially available serum-containing or serum-free media.

The recombinant hybrid proteins expressed using the methods described herein are isolated and purified by

conventional procedures, including separating the cells from the medium by centrifugation or filtration, precipitating the proteinaceous components of the supernatant or filtrate by means of a salt, e.g. ammonium sulfate, purification by a variety of chromatographic procedures, e.g. ion exchange chromatography or affinity chromatography, or the like. Methods of protein purification are known in the art (see generally, Scopes, R., Protein Purification, Springer-Verlag, NY (1982), which is incorporated herein by reference) and may be applied to the purification of the recombinant proteins of the present invention.

The hybrid proteins of the present invention may be used as components of tissue adhesives. It is preferred that the tissue adhesives be formulated to provide a concentration of the hybrid proteins of the present invention of between about 5 mg/ml to 100 mg/ml, with concentrations in the range of 35 to 50 mg/ml being particularly preferred. As disclosed above, tissue adhesives generally contain factor XIII and thrombin. Additional components may also be included in the tissue adhesive formulations. These additional components include growth factors such as PDGF, bFGF, TGF α , or EGF and protease inhibitors, such as aprotinin, transexamic acid, alpha-2 plasmin inhibitor, alpha-1-antitrypsin or the Pittsburgh mutant of alpha-1-antitrypsin (Arg-358 alpha-1-antitrypsin). The tissue adhesives may also contain salts, buffering agents, reducing agents, bulking agents, and solubility enhancers. Albumin, NaCl, CaCl $_2$, citrate and phosphate buffers, for example, may be included. Preferably, the tissue adhesives of the present invention are prepared as lyophilized powders, liquid concentrates of ready-to-use liquids. Lyophilized powders are preferred for ease of handling and storage.

The following examples are offered by way of illustration and not by way of limitation.

EXAMPLES

Example 1 - Subcloning and Modification of ADH2 Promoters

An ADH2-4^C promoter was constructed as described in co-pending U.S. Patent Application 07/631,763, CA 1,304,020 and EP 284 044, which are incorporated herein by reference. A DNA construct comprising the complete ADH2-4^C promoter mutagenized at the 3' end to place an Eco RI site in place of the translation start codon, designated p410-4^C (deposited with the American Type Culture Collection (12301 Parklawn Dr., Rockville, MD 20852) under accession number 68861) was used as the source of the ADH2-4^C promoter.

A PAP-I cDNA (U.S. Patent No. 4,937,324) was joined with the ADH2-4^C promoter. Plasmid pAP1.7, comprising the 1.7 kb cDNA in pUC18, was cut with Nco I and Bam HI, and the linearized plasmid was isolated through two rounds of gel purification. The ADH2-4^C promoter from p410-4^C was joined to the 5' end of the PAP-I cDNA via an Eco RI-Nco I adapter. The 1.2 kb Bam HI-Eco RI promoter fragment from p410-4^C, Eco RI-Nco I adapter and the Nco I-Bam HI linearized pAP1.7 plasmid were ligated. The resultant plasmid was designed pPR1. The presence of the correct promoter fusion was confirmed by DNA sequencing.

A yeast expression vector comprising the ADH2-4^C promoter, the PAP-I cDNA and the TPI1 terminator was constructed. Plasmid pZUC13 (comprising the S. cerevisiae chromosomal LEU2 gene and the origin of replication from S. cerevisiae 2 micron plasmid inserted into pUC13 and constructed in a manner analogous to pZUC12, described in published EP 195,691, using the plasmid pMT212, which is described in published EP 163 529) was cut with Bam HI. Plasmid pPR1 was digested completely digested with Bam HI and partially digested with Sac I to isolate the 2.1 kb ADH2-4^C promoter-PAP-I cDNA fragment. Plasmid pTT1 (described in detail below) was digested with Sac I and

Bam HI to isolate the 0.69 bp TPI1 terminator fragment. The Bam HI-Sac I fragment from pPR1 and the Sac I-Bam HI fragment from pTT1 were ligated with the Bam HI-linearized pZUC13. A plasmid containing the expression unit was
5 designated pZ3.

Example 2 - Subcloning of the TPI1 terminator

The yeast TPI1 terminator fragment was obtained from plasmid p270 described by Murray and Kelly (U.S. Patent 4,766,073, which is incorporated by reference
10 herein in its entirety). Plasmid p270 contains the TPI1 terminator inserted as and Xba I-Bam HI fragment into YEpl3. Alternatively, the TPI1 terminator may be obtained from plasmid pM220 (deposited with American Type Culture
15 Collection as an E. coli RR1 transformant under accession number 39853) by digesting the plasmid with Xba I, and Bam HI and purifying the TPI1 terminator fragment (~700 bp).

The TPI1 terminator was removed from plasmid p270 as a Xba I-Bam HI fragment. This fragment was cloned
20 into pUC19 along with another fragment containing the TPI1 promoter fused to the CAT (chloramphenicol acetyl transferase) gene to obtain a TPI1 terminator fragment with an Eco RV end. The resultant plasmid was designated pCAT. The TPI1 terminator was then cut from pCAT as an
25 Eco RV-Bam HI fragment and cloned into pIC19H (Marsh et al., Gene 32:481-486, 1984) which had been cut with the same enzymes, to obtain pTT1 (disclosed in U.S. Patent No. 4,937,324, which is incorporated herein by reference).

30 Example 3 - Construction of Yeast Vectors pDPOT and pRPOT

Plasmid pDPOT was derived from plasmid pCPOT (ATCC No. 39685) by replacing the 750 bp Sph I-Bam HI fragment of pCPOT containing 2 micron and pBR322 sequences
35 with a 186 bp Sph I-Bam HI fragment derived from the pBR322 tetracycline resistance gene.

Plasmid pRPOT was derived from plasmid pDPOT by replacing the Sph I-Bam HI fragment with a polylinker. Plasmid pDPOT was digested with Sph I and Bam HI to isolate the 10.8 kb fragment. Oligonucleotides ZC1551 and ZC1552 (Sequence ID Nos. 7 and 8) were designed to form an adapter with a Bam HI adhesive end and an Sph I adhesive end flanking Sma I, Sst I and Xho I restriction sites. Oligonucleotides ZC1551 and ZC1552 (Sequence ID Nos. 7 and 8) were kinased and annealed to form the Bam HI-Sph I adapter. The 10.8 kb pDPOT fragment was circularized by ligation with the ZC1551/ZC1552 adapter (Sequence ID Nos. 7 and 8). The resultant plasmid was termed pRPOT.

Example 4 - Construction of a Fibrinogen:Fibronectin Hybrid cDNA Expression Vector

A. Construction of pFN14A

A DNA construct containing a DNA segment encoding the fibronectin cell-binding domain operably linked to the ADH2-4^C promoter in plasmid pUC19 was constructed. The fibronectin coding sequence was obtained from plasmid pFH103 (Dufour et al., Exper. Cell Res. 193: 331-338, 1991). Plasmid pFH103 was digested with Nco I and Xba I to isolate the 4 kb fragment containing the fibronectin coding sequence. Oligonucleotides ZC2052 and ZC2053 (Sequence ID Nos. 9 and 10) were designed to provide, upon annealing, an adapter containing a 5' Eco RI adhesive end, an internal Nco I site, a DNA segment encoding a methionine and amino acids 979 through 981 of Sequence ID Number 2 and a 3' Nco I adhesive end that destroys the Nco I site. Oligonucleotides ZC2052 and ZC2053 (Sequence ID Nos. 9 and 10) were annealed and ligated with the 4 kb Nco I-Xba I fibronectin fragment into Eco RI-Xba I linearized pUC19. The resultant plasmid was designated pFN4.

Plasmid pFN4 was digested with Hind III and Apa I to isolate the 3.3 kb fibronectin fragment. Oligonucleotides ZC2493 and ZC2491 (Sequence ID Nos. 12

and 11) were designed to provide, when annealed, an Apa I-Xba I adapter encoding the amino acids Pro and Phe followed by a stop codon. The oligonucleotides were annealed and combined with the 3.3 kb Hind III-Apa I fragment and Hind III-Xba I linearized pUC19 to form plasmid pFN7. Plasmid pFN7 comprises a DNA segment encoding amino acids 1273-2186 of Sequence ID Number 2 followed by an in-frame stop codon.

The ADH2-4^C promoter was joined to the 5' end of the fibronectin cDNA in plasmid pFN5. Plasmid pFN4 was digested with Nco I and Hind III to isolate the 0.89 kb fibronectin coding sequence. Plasmid pZ3 (described in detail above) was digested with Bam HI and Nco I to isolate the 1.25 kb ADH2-4^C promoter fragment. The 1.25 kb Bam HI-Nco I promoter fragment and the Nco I-Hind III fibronectin coding sequence fragment were ligated to Bam HI-Hind III linearized pUC19 to form plasmid pFN5.

Plasmid pFN5 was digested with Bam HI and Hind III to isolate the 2.1 kb promoter-fibronectin fragment. Plasmid pFN7 was digested with Hind III and Xba I to isolate the 2.8 kb fibronectin fragment that was modified to encode a stop codon following the Pro-Phe sequence. The TPI1 terminator sequence was obtained from pTT1 as a 0.7 kb Xba I-Sal I fragment. The 2.1 kb Bam HI-Hind III promoter-fibronectin fragment, the 2.8 kb Hind III-Xba I fibronectin fragment and the 0.7 kb TPI1 terminator fragment were joined in a four-part ligation with Bam HI-Xho I linearized pRPOT. A plasmid containing the fibronectin expression unit in the pRPOT vector was designated pR1.

The original clone pFH103 contained a frame-shift mutation in the EIIIB region of the fibronectin cDNA. The mutation was corrected by the replacement of the region with an analogous region from the plasmid pFHΔ3 (obtained from Jean Paul Thiery, Laboratoire de Physiopathologie du Developpement, CNRS URA 1337, Ecole Normale Supérieure, 46 rue d'Ulm, 75230 Paris Cedex 05,

France). Plasmid pFHΔ3 was derived from pFH103 by excising the 3211 bp Xba I-Asp 718I fragment of fibronectin, blunting of the resultant adhesive ends and religating. Plasmid pFHΔ3 contains a DNA segment encoding the signal and propeptides, the first three and one half type I repeats, and the carboxy-terminal half of human fibronectin from the middle of the EIIIB segment.

Plasmid pR1 was digested with Bam HI and Kpn I to isolate the 2.2 kb promoter-fibronectin fragment. Plasmid pFHΔ3 was digested with Kpn I and Apa I to isolate the internal fibronectin fragment that corrects the frame-shift mutation present in the parent cDNA from pFH103. Plasmid pR1 was digested with Apa I and Bam HI to isolate the TPI1 terminator fragment. The 2.2 kb Bam HI-Kpn I promoter-fibronectin fragment, the 2.75 kb Kpn I-Apa I internal fibronectin fragment and the 0.69 kb Apa I-Bam HI TPI1 terminator fragment were joined in a four-part ligation with Bam HI-linearized pDPOT. The resulting construction was designated pD32.

A DNA segment encoding the ADH2-4^C promoter and initiation methionine from plasmid pD32 was subcloned into pIC19H (Marsh et al., Gene 32:481-486, 1984) as a 1.25 kb Bam HI-Nco I fragment. Plasmid pD32 was also digested with Nco I and Bgl II to isolate the 3 kb fibronectin cDNA fragment encoding amino acids 979-1972 of Sequence ID Number 2. The 1.25 kb Bam HI-Nco I fragment and the Nco I-Bgl II fragment were ligated with Bam HI-linearized pIC19H. A plasmid containing a Bam HI site proximal to the ADH2-4^C promoter was designated pFN14A.

30

B. - Construction of Plasmid pD38

An expression vector comprising a DNA segment encoding a fibronectin-fibrinogen hybrid protein operably linked to the ADH2-4^C promoter and the TPI1 terminator was constructed. To assemble the DNA sequence encoding the hybrid protein, a DNA segment encoding approximately the

35

carboxy-terminal 409 amino acids of the α chain of fibrinogen was first subcloned.

A fibrinogen α chain cDNA was obtained from Dominic W. Chung (Department of Biochemistry, University of Washington, Seattle, WA) in plasmid pHl α 3 (Rixon et al., Biochemistry 22: 3250-3256, 1983). Sequence analysis of the cDNA insert in plasmid pHl α -3 revealed a deletion of codons 1348-1350 of the published sequence resulting in the deletion of Serine, amino acid 417.

The DNA segment encoding the carboxy-terminus of the fibrinogen α chain was subcloned into plasmid pUC19. Plasmid pHl α -3 was digested with Asp 718 and Ssp I to isolate the approximately 2 kb fragment encoding the carboxy-terminus of the fibrinogen α chain from amino acid 244 to amino acid 643 and some 3' untranslated sequence of Sequence ID Number 4. Plasmid pTT1 was digested with Eco RV and Sal I to isolate the approximately 700 bp TPI1 terminator fragment. The 2 kb fibrinogen α chain sequence and the TPI1 terminator sequence were ligated with pUC19 that had been linearized with Asp 718 and Sal I. The ligation mixture was transformed into E. coli, and plasmid DNA was prepared and analyzed by restriction endonuclease and DNA sequence analysis. DNA sequence analysis of a candidate clone revealed that the Sal I site joining the TPI1 terminator sequence and the pUC19 polylinker site was not present. Plasmid DNA from the candidate clone was digested with Asp 718 and Bam HI to liberate the approximately 1.9 kb fibrinogen-TPI1 terminator fragment.

To join the fibronectin coding sequence with the fibrinogen α chain sequence, synthetic oligonucleotides were synthesized to provide, when annealed, a Sal I-Asp 718 adapter encoding an internal Afl II restriction site, and a sequence encoding amino acids 1886 through 1903 of fibronectin (Sequence ID Number 2), a glycine residue and amino acids 235 through 243 of the fibrinogen α chain (Sequence ID Number 4). Oligonucleotides ZC3521 and ZC3522 (Sequence ID Nos. 13 and 14) were annealed. The

1.9 kb Asp 718-Bam HI fibrinogen-TPI1 terminator fragment and the Sal I-Asp 718 ZC3521/ZC3522 adapter (Sequence ID Nos. 13 and 14) were ligated with pUC19 that had been linearized with Sal I and Bam HI. The resultant plasmid was designated pFG4.

The DNA segment encoding the fibronectin-fibrinogen α chain sequence in plasmid pFG4 was joined with the DNA segment encoding the amino-terminal fibronectin sequence (from amino acid 989 to amino acid 1885 of Sequence ID Number 2) in plasmid pFN14A to construct plasmid pD37. Plasmid pFN14A was digested with Bgl II and Afl II to isolate the approximately 3.9 kb ADH2-4^C promoter-fibronectin fragment. Plasmid pFG4 was digested with Afl II and Bam HI to isolate the approximately 2 kb fibronectin-fibrinogen-TPI1 terminator fragment. The 3.9 kb Bgl II-Afl II fragment and the 2 kb Afl II-Bam HI fragment were ligated with Bam HI-linearized pDPOT. A plasmid with the expression unit inserted with the direction of transcription in the same direction as the POT1 gene in the pDPOT vector was designated pD37.

To place the expression unit present in pD37 in the opposite orientation, such that the direction of transcription of the expression unit was in the opposite direction to that of the POT1 gene, plasmid pD37 was digested with Nco I and Xba I to isolate the approximately 4 kb fibronectin-fibrinogen α chain fragment. Plasmid pFN14A was digested with Bam HI and Nco I to isolate the approximately 1.3 kb ADH2-4^C promoter fragment. Plasmid pTT1 was digested with Bam HI and Xba I to isolate the approximately 700 bp TPI1 terminator fragment. The Bam HI-Nco I ADH2-4^C promoter fragment, the Nco I-Xba I fibronectin-fibrinogen α chain fragment and the Xba I-Bam HI TPI1 terminator fragment were ligated with Bam HI-linearized pDPOT that had been treated with calf alkaline phosphatase to prevent recircularization. A plasmid containing the expression unit in the opposite orientation relative to the POT1 gene was designated pD38. The

nucleotide sequence and deduced amino acid sequence of the DNA segment encoding the fibronectin-fibrinogen hybrid of plasmid pD38 is shown in Sequence ID Number 5. Plasmid pD38 was deposited on December 15, 1992 with the American Type Culture Collection (12301 Parklawn Drive, Rockville, MD) as an E. coli transformant.

Example 5 - Expression of a Fibronectin-Fibrinogen Hybrid Protein in Yeast

Plasmid pD38 was transformed into the Saccharomyces cerevisiae host strain ZM118 (MATa/MAT α ura3/ura3 Atp11::URA3/Atp11::URA3 leu2-3,112/leu2-3,112 bar1/bar1 pep4::URA3/pep4::URA3 [cir^O]) using essentially the method described by Hinnen et al. (Proc. Natl. Acad. Sci. USA 75: 1929-1933, 1978). Transformants were selected for their ability to grow on medium containing glucose as the sole carbon source.

The ZM118[pD38] transformant was scaled up in a 60 liter fermenter to facilitate purification of the hybrid protein. A single ZM118[pD38] colony was selected from a YEPD + Ade + Leu plate (Table 1) and inoculated into -LeuTrpThrD medium (Table 1). The culture was incubated for approximately 52 hours after which the cells were harvested. The cells were washed in T.E. buffer (Sambrook et al., *ibid.*), resuspended in T.E. buffer + 30% glycerol, and aliquotted into 1 ml seed vials. The seed vials were stored at -80°C. One seed vial was used to inoculate 100 ml of YEPD + Ade + Leu (Table 1). The culture was grown for approximately 28 hours to a final A₆₆₀ of 7.7. The 100 ml culture of ZM118[pD38] was inoculated into a 10 liter fermenter with a final volume of 6.0 liters of medium containing 10 g/L (NH₄)₂SO₄, 5 g/L KH₂PO₄, 5 g/L MgSO₄·7H₂O, 1 g/L NaCl, 0.5 g/L CaCl₂·2H₂O, 3.68 g/L A.A.I. (Table 1), 4.2 g/L citric acid, 60 g/L glucose, 10 ml/L Trace Metal Solution (Table 1), 0.4 ml/L PPG-2025 (Polypropylene glycol, MW 2025, Union Carbide Corp, Danbury, CT) that had been pH adjusted to pH 5.0

with NaOH. In addition to the inoculation culture, 30 ml of Vitamin solution was added (Table 1). The culture was grown for 23 hours at 30°C with the addition of 2 M NaOH to maintain pH of approximately 5.

Table 1
Media Recipes

5	<u>-LeuThrTrp Amino Acid Mixture</u>
	4 g adenine
	3 g L-arginine
	5 g L-aspartic acid
	2 g L-histidine free base
10	6 g L-isoleucine
	4 g L-lysine-mono hydrochloride
	2 g L-methionine
	6 g L-phenylalanine
	5 g L-serine
15	5 g L-tyrosine
	4 g uracil
	6 g L-valine

20 Mix all the ingredients and grind with a mortar and pestle until the mixture is finely ground.

	<u>-LeuTrpThrD</u>
	20 g glucose
25	6.7 g Yeast Nitrogen Base without amino acids (DIFCO Laboratories, Detroit, MI)
	0.6 g -LeuThrTrp Amino Acid Mixture
	18 g Agar

30 Mix all the ingredients in distilled water. Add distilled water to a final volume of 1 liter. Autoclave 15 minutes. Pour plates and allow to solidify.

Table 1 continuedYEPD + Ade + Leu Plates

	20 g	glucose	
	20 g	Bacto Peptone (DIFCO Laboratories)	
5	10 g	Bacto Yeast Extract (DIFCO Laboratories)	
	18 g	agar	
	4 ml	1% adenine	
	8 ml	1% L-leucine	

10

Mix all ingredients in distilled water, and bring to a final volume of 1 liter. Autoclave 25 minutes and pour plates.

15

YEPD + Ade + Leu Medium

	20 g	glucose	
	20 g	Bacto Peptone (DIFCO Laboratories)	
	10 g	Bacto Yeast Extract (DIFCO Laboratories)	
20	4 ml	1% adenine	
	8 ml	1% L-leucine	

25

Mix all ingredients in distilled water, and bring to a final volume of 1 liter. Autoclave 25 minutes.

Table 1 continued

	<u>A.A.I.</u>	
	4.0 g	adenine
	5.0 g	L-alanine
5	2.0 g	L-arginine
	5.0 g	L-asparagine
	5.0 g	L-aspartic acid
	5.0 g	L-cysteine
	5.0 g	L-glutamine
10	5.0 g	L-glutamic acid
	5.0 g	L-glycine
	8.0 g	L-histidine
	5.0 g	L-isoleucine
	3.0 g	L-lysine-mono hydrochloride
15	2.0 g	L-methionine
	5.0 g	L-phenylalanine
	5.0 g	L-proline
	5.0 g	L-serine
	5.0 g	L-threonine
20	2.0 g	L-tryptophan
	3.0 g	L-tyrosine
	3.0 g	uracil
	5.0 g	L-valine
25		Mix all the ingredients and grind with a mortar and pestle until the mixture is finely ground. Store at room temperature.

Table 1 continuedTrace Metal Solution

	0.68 g	ZnCl ₂
	5.4 g	FeCl ₃ ·6H ₂ O
5	1.91 g	MnCl ₂ ·4H ₂ O
	0.22 g	CuSO ₄ ·5H ₂ O
	0.258 g	CoCl ₂
	0.062 g	H ₃ BO ₃
	0.002 g	(NH ₄) ₆ Mo ₂ O ₂
10	0.002 g	KI
	10.0 ml	37% HCl

Dissolve solids in water and bring to
a final volume of 1 liter.

15

Vitamin Solution

	25 mg	d-biotin
	400 mg	thiamine
	400 mg	pyridoxine
20	7.5 g	meso-inositol
	7.5 g	Ca pantothenate
	300 mg	niacinamide
	50 mg	folic acid
	100 mg	riboflavin
25	500 mg	choline

Dissolve solids in water and bring to
a final volume of 1 liter.

- 30 A 60 liter fermenter with a final volume of 50
liters of medium containing 60 g/L yeast extract
(Universal Foods, Milwaukee, WI), 2.5 g/L MgSO₄·7H₂O
(Mallinkrodt Inc., St. Louis, MO), 1 g/L CaCl₂·2H₂O
(Mallinkrodt, Inc.), 1 g/L KCl (Mallinkrodt, Inc.), 10
35 ml/L of Trace Metal Solution (Table 1), 0.5 ml/L PPG-2025
(Union Carbide) that had been adjusted to a pH of 5.0 with

H₃PO₄ was prepared, and the medium was sterilized. After sterilization, 5.0 liters of the 23 hour fermentation culture and 500 ml of Vitamin Solution (Table 1) were inoculated into the medium. During the fermentation, a
5 solution of 50% glucose, 5% (NH₄)₂SO₄, 0.05% citric acid was fed into the fermenter at a rate of 150 ml/hour, and the pH was maintained at approximately pH 5 by the addition of 2 M NH₄OH. PPG-2025 was added as needed to control foaming. At approximately 49 hours post
10 inoculation, an ethanol feed was begun by the addition of ethanol to the fermenter at a rate of 150 ml/min. The culture was grown for a total of 67.25 hours at 30°C.

At the end of the fermentation, 50 liters of the culture was diluted to 100 liters with water. The cells
15 were removed from the spent medium by centrifuging 50 liters at a time through a Westfalia CSA 19 centrifuge (Westfalia, Oelde, Germany) at a flow rate of 4 liters/min. The cells were rinsed with water. From the centrifugation, approximately 20 liters of cell slurry
20 containing approximately 35% cells was obtained. Salts were added to the slurry to achieve a final concentration of the following salts: 50 mM NaCl, 10 mM Na₂HPO₄, 5 mM EDTA. The cell slurry was passed through a Dynamill bead mill using 0.5 mm lead-free glass beads (Willy A Bachofen
25 AG MashinenFabrik, Basle, Switzerland) at a rate of 4 liters per minute. The Dynamill was rinsed with Lysis buffer (50 mM NaCl, 10 mM Na₂HPO₄, 5 mM EDTA, pH 7.2) to a final volume of 80 liters. The final slurry had a pH of 6.8, a temperature of approximately 10°C and a
30 conductivity of 5 ms/cm.

The cell slurry was subjected to centrifugation as described above, and the cell pellet was rinsed with lysis buffer. After centrifugation approximately 20
liters of cell slurry was obtained. The cell slurry was
35 extracted by first adjusting the concentration of the cell debris to approximately 40-50% with lysis buffer. Solid urea, NaCl and EDTA were added to the cell slurry to

achieve a final concentration of approximately 8 M urea, 0.3 M NaCl and 10 mM EDTA. The approximate salt concentrations were obtained by the addition of 450 g/L of urea, 18 g/L of NaCl and 4.2 g/L of EDTA. The cell slurry
5 was adjusted to pH 7.8 with 0.5 M NaOH. The solids were dissolved into the slurry and the pellets were extracted for a total of 50 minutes. Following extraction, the mixture was diluted 1 to 4 with water, adjusted to a conductivity of 12.5 ms/cm with NaCl and adjusted to a pH
10 of 9.5 with 0.5 M NaOH.

The extracted slurry was centrifuged as described above with the lysis buffer rinse. The pH of the supernatant was adjusted to pH 9.5 with 0.5 M NaOH. The supernatant was analyzed by SDS polyacrylamide gel
15 electrophoresis (SDS-PAGE) using the PHAST System Separation and Control Unit (Pharmacia LKB Biotechnology Inc., Piscataway, NJ), and the protein was visualized using Coomassie Blue staining. A 2 liter Q-sepharose column (Pharmacia) was equilibrated at 5 liters/hour with
20 successive washes of the following solutions: 8 liters of 3 M urea, 1 M NaCl, 50 mM glycine, pH 11.5; 5 liters of 0.5 M NaOH; 1.5 liters of water; 5 liters of 0.1 M HCl; and 6.0 liters of Wash buffer (50 mM glycine, 90 mM NaCl, pH 9.5 with a conductivity of 12.5 ms/cm). The
25 supernatant (110 liters) was then applied to the column at 5 liters per hour.

The column ran dry after loading the supernatant. The gel was resuspended in Wash buffer and repacked. The repacked column was washed with 4 liters of
30 50 mM glycine, 90 mM NaCl, 5 mM EDTA, pH 10.0. The material was eluted with elution buffer (50 mM glycine, 5 mM EDTA (pH 9.9) with a final concentration of NaCl giving a conductivity of 30.2 cm/ms (approximately 270 mM NaCl)) at 100 ml per minute. The approximately 600 ml fractions
35 were collected after the conductivity of the eluant reached the conductivity of the elution buffer. Fractions

were analyzed by SDS-PAGE analysis as described above and fractions 1 through 10 were pooled.

The pooled fractions were then applied to a 2 liter phenyl Sepharose column (Pharmacia) that had been
5 equilibrated by successive washes at 5 liters per hour with the following solutions: 3 liters of 0.5 M NaOH; 3 liters of water; 3 liters of 2 M urea, 50 mM glycine, pH 10.5; 1.5 liters of water; 3 liters of 0.1 M HCl; and 3
10 liters of Equilibration buffer (50 mM glycine, 2.5 M NaCl, 2 mM EDTA (pH 10.0) with a conductivity of 180 ms/cm). The pooled peak fractions, which had been adjusted to a conductivity of 180 ms/cm with NaCl and a pH of 10.0 with
15 0.5 M NaOH, were loaded onto the phenyl sepharose column. Following the loading of the peak fractions, the column was washed with Equilibration buffer. The column was eluted with 6 liters of 50 mM glycine, 2 mM EDTA (pH
20 10.25) with a NaCl concentration giving the solution a conductivity of 96 ms/cm. The conductivity of the eluant was measured throughout the elution. The conductivity of the eluant upon starting the elution was 180 ms/cm. In the third fraction, the conductivity of the eluant dropped to 96 ms/cm. At this point, the elution buffer was changed to a buffer having the conductivity of 42 ms/cm. The eluant was collected through fraction number 8.

Example 6 - Cross-Linking Assay Using the Hybrid Fibrinogen-Fibronectin Protein

The ability of the purified fibrinogen-fibronectin hybrid protein to form transglutaminase-catalyzed interchain cross links was assessed. The transglutaminase activity was provided by the addition of recombinant factor XIII and thrombin or by the addition of recombinant factor XIIIa.

10 A. Preparation of Factor XIII

Recombinant factor XIII was prepared essentially as described in co-pending U.S. Patent Application No. 07/927,196, which is incorporated by reference herein in its entirety. Briefly, factor XIII was isolated from a strain of the yeast Saccharomyces cerevisiae that had been transformed with an expression vector capable of directing the expression of factor XIII. The factor XIII-producing cells were harvested and lysed, and a cleared lysate was prepared. The lysate was fractionated by anion exchange chromatography at neutral to slightly alkaline pH using a column of derivatized agarose, such as DEAE FAST-FLOW SEPHAROSE (Pharmacia LKB Biotechnology, Piscataway, NJ) or the like. Factor XIII was then precipitated from the column eluate by concentrating the eluate and adjusting the pH to between 5.2 and 5.5, such as by diafiltration against ammonium succinate buffer. The precipitate was then dissolved and further purified using conventional chromatographic techniques, such as gel filtration and hydrophobic interaction chromatography. The purified factor XIII was dialyzed, filtered, aliquotted and lyophilized. The factor XIIIa content was determined (Bishop et al., Biochemistry 29: 1861-1869, 1990, which is incorporated by reference herein in its entirety) by fluorometric assay of the dissolved, thrombin-activated material.

Factor XIII was activated to factor XIIIa by adding 2 U of thrombin per 100 mg of factor XIII. The

factor XIII was dissolved in buffer (20 mM sodium borate (pH 8.3), 1 mM CaCl_2). The thrombin was added, and the reaction was incubated at room temperature for twenty minutes.

5

B. Cross-Linking Assays

The level of cross-linking between the hybrid proteins was measured as a rise in the absorbance at 350 nm over time in reaction mixtures containing the hybrid protein, factor XIII and thrombin or the hybrid protein and factor XIIIa. Control reactions were prepared containing factor XIII and thrombin or factor XIIIa alone. Cross-linking reactions were carried out in 1 ml cuvettes. For cross-linking reactions containing factor XIII and thrombin, each reaction mixture was set up by placing 110 μl containing 40 Units of factor XIII, 36.7 μl containing 13 Units of factor XIII or 12.2 μl containing 4 Units of factor XIII (described above) in one corner of the cuvette and 20 μl containing 4 Units of thrombin (Sigma) in the opposite corner such that the solutions were not mixed. The reaction was initiated by the addition of 1 ml of 2 mg/ml hybrid protein in buffer (10 mM Tris (pH 7.6), 20 mM sodium borate, 140 mM NaCl, 10 mM CaCl_2). The absorbance of each reaction was read at 350 nm with the addition of protein being the first absorbance point. For cross-linking reactions containing factor XIIIa, each reaction was set up by placing 110 μl containing 40 Units of factor XIIIa, 36.7 μl containing 13 Units of factor XIIIa or 12.2 μl containing 4 Units of factor XIIIa in the cuvette and adding 1 ml of 2 mg/ml hybrid in buffer (10 mM Tris (pH 7.6), 140 mM NaCl, 10 mM CaCl_2). The absorbance of the solution was read at 350 nm as described above. Analysis of the data generated from the absorbance time courses showed a sharp increase in absorbance in the presence of hybrid protein and the active transglutaminase relative to the rise in absorbance in the absence of hybrid protein

(Figures 2-5). The results indicated that the hybrid protein is capable of transglutaminase-induced cross-linking.

5 From the foregoing it will be appreciated that, although specific embodiments of the invention have been described herein for the purpose of illustration, various modifications may be made without deviation from the spirit and scope of the invention. Accordingly, the invention is not to be limited except as by the following
10 claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Irani, Meher H.
- (ii) TITLE OF INVENTION: HYBRID CROSS-LINKING PROTEINS
- (iii) NUMBER OF SEQUENCES: 14
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: ZymoGenetics, Inc.
 - (B) STREET: 4225 Roosevelt Way, N.E.
 - (C) CITY: Seattle
 - (D) STATE: WA
 - (E) COUNTRY: USA
 - (F) ZIP: 98105
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: WO
 - (B) FILING DATE:
 - (C) CLASSIFICATION:
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 - (A) APPLICATION NUMBER: US 07/998,271
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- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Parker, Gary E
 - (B) REGISTRATION NUMBER: 31-648
 - (C) REFERENCE/DOCKET NUMBER: 92-26PC
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: 206-547-8080 ext 322
 - (B) TELEFAX: 206-548-2329

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 7803 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ix) FEATURE:
 - (A) NAME/KEY: CDS

(B) LOCATION: 6..7346

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TCAAC ATG CTT AGG GGT CCG GGG CCC GGG CTG CTG CTG CTG GCC GTC	47
Met Leu Arg Gly Pro Gly Pro Gly Leu Leu Leu Leu Ala Val	
1 5 10	
CTG TGC CTG GGG ACA GCG GTG CCC TCC ACG GGA GCC TCG AAG AGC AAG	95
Leu Cys Leu Gly Thr Ala Val Pro Ser Thr Gly Ala Ser Lys Ser Lys	
15 20 25 30	
AGG CAG GCT CAG CAA ATG GTT CAG CCC CAG TCC CCG GTG GCT GTC AGT	143
Arg Gln Ala Gln Gln Met Val Gln Pro Gln Ser Pro Val Ala Val Ser	
35 40 45	
CAA AGC AAG CCC GGT TGT TAT GAC AAT GGA AAA CAC TAT CAG ATA AAT	191
Gln Ser Lys Pro Gly Cys Tyr Asp Asn Gly Lys His Tyr Gln Ile Asn	
50 55 60	
CAA CAG TGG GAG CGG ACC TAC CTA GGT AAT GTG TTG GTT TGT ACT TGT	239
Gln Gln Trp Glu Arg Thr Tyr Leu Gly Asn Val Leu Val Cys Thr Cys	
65 70 75	
TAT GGA GGA AGC CGA GGT TTT AAC TGC GAA AGT AAA CCT GAA GCT GAA	287
Tyr Gly Gly Ser Arg Gly Phe Asn Cys Glu Ser Lys Pro Glu Ala Glu	
80 85 90	
GAG ACT TGC TTT GAC AAG TAC ACT GGG AAC ACT TAC CGA GTG GGT GAC	335
Glu Thr Cys Phe Asp Lys Tyr Thr Gly Asn Thr Tyr Arg Val Gly Asp	
95 100 105 110	
ACT TAT GAG CGT CCT AAA GAC TCC ATG ATC TGG GAC TGT ACC TGC ATC	383
Thr Tyr Glu Arg Pro Lys Asp Ser Met Ile Trp Asp Cys Thr Cys Ile	
115 120 125	
GGG GCT GGG CGA GGG AGA ATA AGC TGT ACC ATC GCA AAC CGC TGC CAT	431
Gly Ala Gly Arg Gly Arg Ile Ser Cys Thr Ile Ala Asn Arg Cys His	
130 135 140	
GAA GGG GGT CAG TCC TAC AAG ATT GGT GAC ACC TGG AGG AGA CCA CAT	479
Glu Gly Gly Gln Ser Tyr Lys Ile Gly Asp Thr Trp Arg Arg Pro His	
145 150 155	
GAG ACT GGT GGT TAC ATG TTA GAG TGT GTG TGT CTT GGT AAT GGA AAA	527
Glu Thr Gly Gly Tyr Met Leu Glu Cys Val Cys Leu Gly Asn Gly Lys	
160 165 170	
GGA GAA TGG ACC TGC AAG CCC ATA GCT GAG AAG TGT TTT GAT CAT GCT	575
Gly Glu Trp Thr Cys Lys Pro Ile Ala Glu Lys Cys Phe Asp His Ala	
175 180 185 190	
GCT GGG ACT TCC TAT GTG GTC GGA GAA ACG TGG GAG AAG CCC TAC CAA	623
Ala Gly Thr Ser Tyr Val Val Gly Glu Thr Trp Glu Lys Pro Tyr Gln	
195 200 205	

GGC	TGG	ATG	ATG	GTA	GAT	TGT	ACT	TGC	CTG	GGA	GAA	GGC	AGC	GGA	CGC	671
Gly	Trp	Met	Met	Val	Asp	Cys	Thr	Cys	Leu	Gly	Glu	Gly	Ser	Gly	Arg	
			210					215					220			
ATC	ACT	TGC	ACT	TCT	AGA	AAT	AGA	TGC	AAC	GAT	CAG	GAC	ACA	AGG	ACA	719
Ile	Thr	Cys	Thr	Ser	Arg	Asn	Arg	Cys	Asn	Asp	Gln	Asp	Thr	Arg	Thr	
		225					230					235				
TCC	TAT	AGA	ATT	GGA	GAC	ACC	TGG	AGC	AAG	AAG	GAT	AAT	CGA	GGA	AAC	767
Ser	Tyr	Arg	Ile	Gly	Asp	Thr	Trp	Ser	Lys	Lys	Asp	Asn	Arg	Gly	Asn	
	240					245					250					
CTG	CTC	CAG	TGC	ATC	TGC	ACA	GGC	AAC	GGC	CGA	GGA	GAG	TGG	AAG	TGT	815
Leu	Leu	Gln	Cys	Ile	Cys	Thr	Gly	Asn	Gly	Arg	Gly	Glu	Trp	Lys	Cys	
255					260					265					270	
GAG	AGG	CAC	ACC	TCT	GTG	CAG	ACC	ACA	TCG	AGC	GGA	TCT	GGC	CCC	TTC	863
Glu	Arg	His	Thr	Ser	Val	Gln	Thr	Thr	Ser	Ser	Gly	Ser	Gly	Pro	Phe	
				275					280					285		
ACC	GAT	GTT	CGT	GCA	GCT	GTT	TAC	CAA	CCG	CAG	CCT	CAC	CCC	CAG	CCT	911
Thr	Asp	Val	Arg	Ala	Ala	Val	Tyr	Gln	Pro	Gln	Pro	His	Pro	Gln	Pro	
			290					295					300			
CCT	CCC	TAT	GGC	CAC	TGT	GTC	ACA	GAC	AGT	GGT	GTG	GTC	TAC	TCT	GTG	959
Pro	Pro	Tyr	Gly	His	Cys	Val	Thr	Asp	Ser	Gly	Val	Val	Tyr	Ser	Val	
		305					310					315				
GGG	ATG	CAG	TGG	TTG	AAG	ACA	CAA	GGA	AAT	AAG	CAA	ATG	CTT	TGC	ACG	1007
Gly	Met	Gln	Trp	Leu	Lys	Thr	Gln	Gly	Asn	Lys	Gln	Met	Leu	Cys	Thr	
	320					325					330					
TGC	CTG	GGC	AAC	GGA	GTC	AGC	TGC	CAA	GAG	ACA	GCT	GTA	ACC	CAG	ACT	1055
Cys	Leu	Gly	Asn	Gly	Val	Ser	Cys	Gln	Glu	Thr	Ala	Val	Thr	Gln	Thr	
335					340					345					350	
TAC	GGT	GGC	AAC	TTA	AAT	GGA	GAG	CCA	TGT	GTC	TTA	CCA	TTC	ACC	TAC	1103
Tyr	Gly	Gly	Asn	Leu	Asn	Gly	Glu	Pro	Cys	Val	Leu	Pro	Phe	Thr	Tyr	
				355					360					365		
AAT	GGC	AGG	ACG	TTC	TAC	TCC	TGC	ACC	ACG	GAA	GGG	CGA	CAG	GAC	GGA	1151
Asn	Gly	Arg	Thr	Phe	Tyr	Ser	Cys	Thr	Thr	Glu	Gly	Arg	Gln	Asp	Gly	
			370					375					380			
CAT	CTT	TGG	TGC	AGC	ACA	ACT	TCG	AAT	TAT	GAG	CAG	GAC	CAG	AAA	TAC	1199
His	Leu	Trp	Cys	Ser	Thr	Thr	Ser	Asn	Tyr	Glu	Gln	Asp	Gln	Lys	Tyr	
		385					390					395				
TCT	TTC	TGC	ACA	GAC	CAC	ACT	GTT	TTG	GTT	CAG	ACT	CAA	GGA	GGA	AAT	1247
Ser	Phe	Cys	Thr	Asp	His	Thr	Val	Leu	Val	Gln	Thr	Gln	Gly	Gly	Asn	
	400					405					410					
TCC	AAT	GGT	GCC	TTG	TGC	CAC	TTC	CCC	TTC	CTA	TAC	AAC	AAC	CAC	AAT	1295
Ser	Asn	Gly	Ala	Leu	Cys	His	Phe	Pro	Phe	Leu	Tyr	Asn	Asn	His	Asn	
415					420					425					430	

TAC	ACT	GAT	TGC	ACT	TCT	GAG	GGC	AGA	AGA	GAC	AAC	ATG	AAG	TGG	TGT	1343
Tyr	Thr	Asp	Cys	Thr	Ser	Glu	Gly	Arg	Arg	Asp	Asn	Met	Lys	Trp	Cys	
				435					440					445		
GGG	ACC	ACA	CAG	AAC	TAT	GAT	GCC	GAC	CAG	AAG	TTT	GGG	TTC	TGC	CCC	1391
Gly	Thr	Thr	Gln	Asn	Tyr	Asp	Ala	Asp	Gln	Lys	Phe	Gly	Phe	Cys	Pro	
			450					455					460			
ATG	GCT	GCC	CAC	GAG	GAA	ATC	TGC	ACA	ACC	AAT	GAA	GGG	GTC	ATG	TAC	1439
Met	Ala	Ala	His	Glu	Glu	Ile	Cys	Thr	Thr	Asn	Glu	Gly	Val	Met	Tyr	
		465					470					475				
CGC	ATT	GGA	GAT	CAG	TGG	GAT	AAG	CAG	CAT	GAC	ATG	GGT	CAC	ATG	ATG	1487
Arg	Ile	Gly	Asp	Gln	Trp	Asp	Lys	Gln	His	Asp	Met	Gly	His	Met	Met	
	480					485					490					
AGG	TGC	ACG	TGT	GTT	GGG	AAT	GGT	CGT	GGG	GAA	TGG	ACA	TGC	ATT	GCC	1535
Arg	Cys	Thr	Cys	Val	Gly	Asn	Gly	Arg	Gly	Glu	Trp	Thr	Cys	Ile	Ala	
495					500					505					510	
TAC	TCG	CAA	CTT	CGA	GAT	CAG	TGC	ATT	GTT	GAT	GAC	ATC	ACT	TAC	AAT	1583
Tyr	Ser	Gln	Leu	Arg	Asp	Gln	Cys	Ile	Val	Asp	Asp	Ile	Thr	Tyr	Asn	
				515					520					525		
GTG	AAC	GAC	ACA	TTC	CAC	AAG	CGT	CAT	GAA	GAG	GGG	CAC	ATG	CTG	AAC	1631
Val	Asn	Asp	Thr	Phe	His	Lys	Arg	His	Glu	Glu	Gly	His	Met	Leu	Asn	
			530					535					540			
TGT	ACA	TGC	TTC	GGT	CAG	GGT	CGG	GGC	AGG	TGG	AAG	TGT	GAT	CCC	GTC	1679
Cys	Thr	Cys	Phe	Gly	Gln	Gly	Arg	Gly	Arg	Trp	Lys	Cys	Asp	Pro	Val	
		545					550					555				
GAC	CAA	TGC	CAG	GAT	TCA	GAG	ACT	GGG	ACG	TTT	TAT	CAA	ATT	GGA	GAT	1727
Asp	Gln	Cys	Gln	Asp	Ser	Glu	Thr	Gly	Thr	Phe	Tyr	Gln	Ile	Gly	Asp	
	560					565					570					
TCA	TGG	GAG	AAG	TAT	GTG	CAT	GGT	GTC	AGA	TAC	CAG	TGC	TAC	TGC	TAT	1775
Ser	Trp	Glu	Lys	Tyr	Val	His	Gly	Val	Arg	Tyr	Gln	Cys	Tyr	Cys	Tyr	
575					580				585						590	
GGC	CGT	GGC	ATT	GGG	GAG	TGG	CAT	TGC	CAA	CCT	TTA	CAG	ACC	TAT	CCA	1823
Gly	Arg	Gly	Ile	Gly	Glu	Trp	His	Cys	Gln	Pro	Leu	Gln	Thr	Tyr	Pro	
				595					600					605		
AGC	TCA	AGT	GGT	CCT	GTC	GAA	GTA	TTT	ATC	ACT	GAG	ACT	CCG	AGT	CAG	1871
Ser	Ser	Ser	Gly	Pro	Val	Glu	Val	Phe	Ile	Thr	Glu	Thr	Pro	Ser	Gln	
			610					615					620			
CCC	AAC	TCC	CAC	CCC	ATC	CAG	TGG	AAT	GCA	CCA	CAG	CCA	TCT	CAC	ATT	1919
Pro	Asn	Ser	His	Pro	Ile	Gln	Trp	Asn	Ala	Pro	Gln	Pro	Ser	His	Ile	
		625					630					635				

TCC Ser 640	AAG Lys	TAC Tyr	ATT Ile	CTC Leu	AGG Arg	TGG Trp 645	AGA Arg	CCT Pro	AAA Lys	AAT Asn 650	TCT Ser	GTA Val	GGC Gly	CGT Arg	TGG Trp	1967
AAG Lys 655	GAA Glu	GCT Ala	ACC Thr	ATA Ile	CCA Pro 660	GGC Gly	CAC His	TTA Leu	AAC Asn 665	TCC Ser	TAC Tyr	ACC Thr	ATC Ile	AAA Lys	GGC Gly 670	2015
CTG Leu	AAG Lys	CCT Pro	GGT Gly	GTG Val 675	GTA Val	TAC Tyr	GAG Glu	GGC Gly	CAG Gln 680	CTC Leu	ATC Ile	AGC Ser	ATC Ile	CAG Gln 685	CAG Gln	2063
TAC Tyr	GGC Gly	CAC His	CAA Gln 690	GAA Glu	GTG Val	ACT Thr	CGC Arg	TTT Phe 695	GAC Asp	TTC Phe	ACC Thr	ACC Thr	ACC Thr	AGC Ser	ACC Thr	2111
AGC Ser	ACA Thr	CCT Pro 705	GTG Val	ACC Thr	AGC Ser	AAC Asn	ACC Thr 710	GTG Val	ACA Thr	GGA Gly	GAG Glu	ACG Thr	ACT Thr	CCC Pro	TTT Phe	2159
TCT Ser 720	CCT Pro	CTT Leu	GTG Val	GCC Ala	ACT Thr	TCT Ser 725	GAA Glu	TCT Ser	GTG Val	ACC Thr	GAA Glu 730	ATC Ile	ACA Thr	GCC Ala	AGT Ser	2207
AGC Ser 735	TTT Phe	GTG Val	GTC Val	TCC Ser	TGG Trp 740	GTC Val	TCA Ser	GCT Ala	TCC Ser	GAC Asp 745	ACC Thr	GTG Val	TCG Ser	GGA Gly	TTC Phe 750	2255
CGG Arg	GTG Val	GAA Glu	TAT Tyr	GAG Glu 755	CTG Leu	AGT Ser	GAG Glu	GAG Glu	GGA Gly 760	GAT Asp	GAG Glu	CCA Pro	CAG Gln 765	TAC Tyr	CTG Leu	2303
GAT Asp	CTT Leu	CCA Pro	AGC Ser 770	ACA Thr	GCC Ala	ACT Thr	TCT Ser	GTG Val 775	AAC Asn	ATC Ile	CCT Pro	GAC Asp	CTG Leu 780	CTT Leu	CCT Pro	2351
GGC Gly	CGA Arg	AAA Lys 785	TAC Tyr	ATT Ile	GTA Val	AAT Asn	GTC Val 790	TAT Tyr	CAG Gln	ATA Ile	TCT Ser	GAG Glu 795	GAT Asp	GGG Gly	GAG Glu	2399
CAG Gln 800	AGT Ser	TTG Leu	ATC Ile	CTG Leu	TCT Ser	ACT Thr 805	TCA Ser	CAA Gln	ACA Thr	ACA Thr	GCG Ala 810	CCT Pro	GAT Asp	GCC Ala	CCT Pro	2447
CCT Pro 815	GAC Asp	CCG Pro	ACT Thr	GTG Val	GAC Asp 820	CAA Gln	GTT Val	GAT Asp	GAC Asp	ACC Thr 825	TCA Ser	ATT Ile	GTT Val	GTT Val	CGC Arg 830	2495
TGG Trp	AGC Ser	AGA Arg	CCC Pro	CAG Gln 835	GCT Ala	CCC Pro	ATC Ile	ACA Thr	GGG Gly 840	TAC Tyr	AGA Arg	ATA Ile	GTC Val	TAT Tyr	TCG Ser	2543
CCA Pro	TCA Ser	GTA Val	GAA Glu 850	GGT Gly	AGC Ser	AGC Ser	ACA Thr	GAA Glu 855	CTC Leu	AAC Asn	CTT Leu	CCT Pro	GAA Glu 860	ACT Thr	GCA Ala	2591

AAC	TCC	GTC	ACC	CTC	AGT	GAC	TTG	CAA	CCT	GGT	GTT	CAG	TAT	AAC	ATC	2639
Asn	Ser	Val	Thr	Leu	Ser	Asp	Leu	Gln	Pro	Gly	Val	Gln	Tyr	Asn	Ile	
		865					870					875				
ACT	ATC	TAT	GCT	GTG	GAA	GAA	AAT	CAA	GAA	AGT	ACA	CCT	GTT	GTC	ATT	2687
Thr	Ile	Tyr	Ala	Val	Glu	Glu	Asn	Gln	Glu	Ser	Thr	Pro	Val	Val	Ile	
	880					885					890					
CAA	CAA	GAA	ACC	ACT	GGC	ACC	CCA	CGC	TCA	GAT	ACA	GTG	CCC	TCT	CCC	2735
Gln	Gln	Glu	Thr	Thr	Gly	Thr	Pro	Arg	Ser	Asp	Thr	Val	Pro	Ser	Pro	
895					900					905					910	
AGG	GAC	CTG	CAG	TTT	GTG	GAA	GTG	ACA	GAC	GTG	AAG	GTC	ACC	ATC	ATG	2783
Arg	Asp	Leu	Gln	Phe	Val	Glu	Val	Thr	Asp	Val	Lys	Val	Thr	Ile	Met	
				915					920					925		
TGG	ACA	CCG	CCT	GAG	AGT	GCA	GTG	ACC	GGC	TAC	CGT	GTG	GAT	GTG	ATC	2831
Trp	Thr	Pro	Pro	Glu	Ser	Ala	Val	Thr	Gly	Tyr	Arg	Val	Asp	Val	Ile	
			930					935					940			
CCC	GTC	AAC	CTG	CCT	GGC	GAG	CAC	GGG	CAG	AGG	CTG	CCC	ATC	AGC	AGG	2879
Pro	Val	Asn	Leu	Pro	Gly	Glu	His	Gly	Gln	Arg	Leu	Pro	Ile	Ser	Arg	
		945					950					955				
AAC	ACC	TTT	GCA	GAA	GTC	ACC	GGG	CTG	TCC	CCT	GGG	GTC	ACC	TAT	TAC	2927
Asn	Thr	Phe	Ala	Glu	Val	Thr	Gly	Leu	Ser	Pro	Gly	Val	Thr	Tyr	Tyr	
	960					965					970					
TTC	AAA	GTC	TTT	GCA	GTG	AGC	CAT	GGG	AGG	GAG	AGC	AAG	CCT	CTG	ACT	2975
Phe	Lys	Val	Phe	Ala	Val	Ser	His	Gly	Arg	Glu	Ser	Lys	Pro	Leu	Thr	
975					980					985					990	
GCT	CAA	CAG	ACA	ACC	AAA	CTG	GAT	GCT	CCC	ACT	AAC	CTC	CAG	TTT	GTC	3023
Ala	Gln	Gln	Thr	Thr	Lys	Leu	Asp	Ala	Pro	Thr	Asn	Leu	Gln	Phe	Val	
				995					1000					1005		
AAT	GAA	ACT	GAT	TCT	ACT	GTC	CTG	GTG	AGA	TGG	ACT	CCA	CCT	CGG	GCC	3071
Asn	Glu	Thr	Asp	Ser	Thr	Val	Leu	Val	Arg	Trp	Thr	Pro	Pro	Arg	Ala	
			1010					1015					1020			
CAG	ATA	ACA	GGA	TAC	CGA	CTG	ACC	GTG	GGC	CTT	ACC	CGA	AGA	GGC	CAG	3119
Gln	Ile	Thr	Gly	Tyr	Arg	Leu	Thr	Val	Gly	Leu	Thr	Arg	Arg	Gly	Gln	
		1025					1030					1035				
CCC	AGG	CAG	TAC	AAT	GTG	GGT	CCC	TCT	GTC	TCC	AAG	TAC	CCC	CTG	AGG	3167
Pro	Arg	Gln	Tyr	Asn	Val	Gly	Pro	Ser	Val	Ser	Lys	Tyr	Pro	Leu	Arg	
	1040					1045					1050					
AAT	CTG	CAG	CCT	GCA	TCT	GAG	TAC	ACC	GTA	TCC	CTC	GTG	GCC	ATA	AAG	3215
Asn	Leu	Gln	Pro	Ala	Ser	Glu	Tyr	Thr	Val	Ser	Leu	Val	Ala	Ile	Lys	
1055					1060					1065					1070	

GGC AAC CAA GAG AGC CCC AAA GCC ACT GGA GTC TTT ACC ACA CTG CAG Gly Asn Gln Glu Ser Pro Lys Ala Thr Gly Val Phe Thr Thr Leu Gln 1075 1080 1085	3263
CCT GGG AGC TCT ATT CCA CCT TAC AAC ACC GAG GTG ACT GAG ACC ACC Pro Gly Ser Ser Ile Pro Pro Tyr Asn Thr Glu Val Thr Glu Thr Thr 1090 1095 1100	3311
ATC GTG ATC ACA TGG ACG CCT GCT CCA AGA ATT GGT TTT AAG CTG GGT Ile Val Ile Thr Trp Thr Pro Ala Pro Arg Ile Gly Phe Lys Leu Gly 1105 1110 1115	3359
GTA CGA CCA AGC CAG GGA GGA GAG GCA CCA CGA GAA GTG ACT TCA GAC Val Arg Pro Ser Gln Gly Gly Glu Ala Pro Arg Glu Val Thr Ser Asp 1120 1125 1130	3407
TCA GGA AGC ATC GTT GTG TCC GGC TTG ACT CCA GGA GTA GAA TAC GTC Ser Gly Ser Ile Val Val Ser Gly Leu Thr Pro Gly Val Glu Tyr Val 1135 1140 1145 1150	3455
TAC ACC ATC CAA GTC CTG AGA GAT GGA CAG GAA AGA GAT GCG CCA ATT Tyr Thr Ile Gln Val Leu Arg Asp Gly Gln Glu Arg Asp Ala Pro Ile 1155 1160 1165	3503
GTA AAC AAA GTG GTG ACA CCA TTG TCT CCA CCA ACA AAC TTG CAT CTG Val Asn Lys Val Val Thr Pro Leu Ser Pro Pro Thr Asn Leu His Leu 1170 1175 1180	3551
GAG GCA AAC CCT GAC ACT GGA GTG CTC ACA GTC TCC TGG GAG AGG AGC Glu Ala Asn Pro Asp Thr Gly Val Leu Thr Val Ser Trp Glu Arg Ser 1185 1190 1195	3599
ACC ACC CCA GAC ATT ACT GGT TAT AGA ATT ACC ACA ACC CCT ACA AAC Thr Thr Pro Asp Ile Thr Gly Tyr Arg Ile Thr Thr Thr Pro Thr Asn 1200 1205 1210	3647
GGC CAG CAG GGA AAT TCT TTG GAA GAA GTG GTC CAT GCT GAT CAG AGC Gly Gln Gln Gly Asn Ser Leu Glu Glu Val Val His Ala Asp Gln Ser 1215 1220 1225 1230	3695
TCC TGC ACT TTT GAT AAC CTG AGT CCC GGC CTG GAG TAC AAT GTC AGT Ser Cys Thr Phe Asp Asn Leu Ser Pro Gly Leu Glu Tyr Asn Val Ser 1235 1240 1245	3743
GTT TAC ACT GTC AAG GAT GAC AAG GAA AGT GTC CCT ATC TCT GAT ACC Val Tyr Thr Val Lys Asp Asp Lys Glu Ser Val Pro Ile Ser Asp Thr 1250 1255 1260	3791
ATC ATC CCA GAG GTG CCC CAA CTC ACT GAC CTA AGC TTT GTT GAT ATA Ile Ile Pro Glu Val Pro Gln Leu Thr Asp Leu Ser Phe Val Asp Ile 1265 1270 1275	3839
ACC GAT TCA AGC ATC GGC CTG AGG TGG ACC CCG CTA AAC TCT TCC ACC Thr Asp Ser Ser Ile Gly Leu Arg Trp Thr Pro Leu Asn Ser Ser Thr 1280 1285 1290	3887

ATT ATT GGG TAC CGC ATC ACA GTA GTT GCG GCA GGA GAA GGT ATC CCT Ile Ile Gly Tyr Arg Ile Thr Val Val Ala Ala Gly Glu Gly Ile Pro 1295 1300 1305 1310	3935
ATT TTT GAA GAT TTT GTG TAC TCC TCA GTA GGA TAC TAC ACA GTC ACA Ile Phe Glu Asp Phe Val Tyr Ser Ser Val Gly Tyr Tyr Thr Val Thr 1315 1320 1325	3983
GGG CTG GAG CCG GGC ATT GAC TAT GAT ATC AGC GTT ATC ACT CTC ATT Gly Leu Glu Pro Gly Ile Asp Tyr Asp Ile Ser Val Ile Thr Leu Ile 1330 1335 1340	4031
AAT GGC GGC GAG AGT GCC CCT ACT ACA CTG ACA CAA CAA ACG GCT GTT Asn Gly Gly Glu Ser Ala Pro Thr Thr Leu Thr Gln Gln Thr Ala Val 1345 1350 1355	4079
CCT CCT CCC ACT GAC CTG CGA TTC ACC AAC ATT GGT CCA GAC ACC ATG Pro Pro Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met 1360 1365 1370	4127
CGT GTC ACC TGG GCT CCA CCC CCA TCC ATT GAT TTA ACC AAC TTC CTG Arg Val Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu Thr Asn Phe Leu 1375 1380 1385 1390	4175
GTG CGT TAC TCA CCT GTG AAA AAT GAG GAA GAT GTT GCA GAG TTG TCA Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp Val Ala Glu Leu Ser 1395 1400 1405	4223
ATT TCT CCT TCA GAC AAT GCA GTG GTC TTA ACA AAT CTC CTG CCT GGT Ile Ser Pro Ser Asp Asn Ala Val Val Leu Thr Asn Leu Leu Pro Gly 1410 1415 1420	4271
ACA GAA TAT GTA GTG AGT GTC TCC AGT GTC TAC GAA CAA CAT GAG AGC Thr Glu Tyr Val Val Ser Val Ser Ser Val Tyr Glu Gln His Glu Ser 1425 1430 1435	4319
ACA CCT CTT AGA GGA AGA CAG AAA ACA GGT CTT GAT TCC CCA ACT GGC Thr Pro Leu Arg Gly Arg Gln Lys Thr Gly Leu Asp Ser Pro Thr Gly 1440 1445 1450	4367
ATT GAC TTT TCT GAT ATT ACT GCC AAC TCT TTT ACT GTG CAC TGG ATT Ile Asp Phe Ser Asp Ile Thr Ala Asn Ser Phe Thr Val His Trp Ile 1455 1460 1465 1470	4415
GCT CCT CGA GCC ACC ATC ACT GGC TAC AGG ATC CGC CAT CAT CCC GAG Ala Pro Arg Ala Thr Ile Thr Gly Tyr Arg Ile Arg His His Pro Glu 1475 1480 1485	4463
CAC TTC AGT GGG AGA CCT CGA GAA GAT CGG GTG CCC CAC TCT CGG AAT His Phe Ser Gly Arg Pro Arg Glu Asp Arg Val Pro His Ser Arg Asn 1490 1495 1500	4511

TCC	ATC	ACC	CTC	ACC	AAC	CTC	ACT	CCA	GGC	ACA	GAG	TAT	GTG	GTC	AGC	4559
Ser	Ile	Thr	Leu	Thr	Asn	Leu	Thr	Pro	Gly	Thr	Glu	Tyr	Val	Val	Ser	
		1505					1510					1515				
ATC	GTT	GCT	CTT	AAT	GGC	AGA	GAG	GAA	AGT	CCC	TTA	TTG	ATT	GGC	CAA	4607
Ile	Val	Ala	Leu	Asn	Gly	Arg	Glu	Glu	Ser	Pro	Leu	Leu	Ile	Gly	Gln	
	1520					1525					1530					
CAA	TCA	ACA	GTT	TCT	GAT	GTT	CCG	AGG	GAC	CTG	GAA	GTT	GTT	GCT	GCG	4655
Gln	Ser	Thr	Val	Ser	Asp	Val	Pro	Arg	Asp	Leu	Glu	Val	Val	Ala	Ala	
1535					1540					1545					1550	
ACC	CCC	ACC	AGC	CTA	CTG	ATC	AGC	TGG	GAT	GCT	CCT	GCT	GTC	ACA	GTG	4703
Thr	Pro	Thr	Ser	Leu	Leu	Ile	Ser	Trp	Asp	Ala	Pro	Ala	Val	Thr	Val	
				1555					1560					1565		
AGA	TAT	TAC	AGG	ATC	ACT	TAC	GGA	GAA	ACA	GGA	GGA	AAT	AGC	CCT	GTC	4751
Arg	Tyr	Tyr	Arg	Ile	Thr	Tyr	Gly	Glu	Thr	Gly	Gly	Asn	Ser	Pro	Val	
			1570					1575					1580			
CAG	GAG	TTC	ACT	GTG	CCT	GGG	AGC	AAG	TCT	ACA	GCT	ACC	ATC	AGC	GGC	4799
Gln	Glu	Phe	Thr	Val	Pro	Gly	Ser	Lys	Ser	Thr	Ala	Thr	Ile	Ser	Gly	
		1585					1590					1595				
CTT	AAA	CCT	GGA	GTT	GAT	TAT	ACC	ATC	ACT	GTG	TAT	GCT	GTC	ACT	GGC	4847
Leu	Lys	Pro	Gly	Val	Asp	Tyr	Thr	Ile	Thr	Val	Tyr	Ala	Val	Thr	Gly	
	1600						1605				1610					
CGT	GGA	GAC	AGC	CCC	GCA	AGC	AGC	AAG	CCA	ATT	TCC	ATT	AAT	TAC	CGA	4895
Arg	Gly	Asp	Ser	Pro	Ala	Ser	Ser	Lys	Pro	Ile	Ser	Ile	Asn	Tyr	Arg	
1615					1620					1625					1630	
ACA	GAA	ATT	GAC	AAA	CCA	TCC	CAG	ATG	CAA	GTG	ACC	GAT	GTT	CAG	GAC	4943
Thr	Glu	Ile	Asp	Lys	Pro	Ser	Gln	Met	Gln	Val	Thr	Asp	Val	Gln	Asp	
				1635					1640					1645		
AAC	AGC	ATT	AGT	GTC	AAG	TGG	CTG	CCT	TCA	AGT	TCC	CCT	GTT	ACT	GGT	4991
Asn	Ser	Ile	Ser	Val	Lys	Trp	Leu	Pro	Ser	Ser	Ser	Pro	Val	Thr	Gly	
			1650					1655					1660			
TAC	AGA	GTA	ACC	ACC	ACT	CCC	AAA	AAT	GGA	CCA	GGA	CCA	ACA	AAA	ACT	5039
Tyr	Arg	Val	Thr	Thr	Thr	Pro	Lys	Asn	Gly	Pro	Gly	Pro	Thr	Lys	Thr	
		1665					1670					1675				
AAA	ACT	GCA	GGT	CCA	GAT	CAA	ACA	GAA	ATG	ACT	ATT	GAA	GGC	TTG	CAG	5087
Lys	Thr	Ala	Gly	Pro	Asp	Gln	Thr	Glu	Met	Thr	Ile	Glu	Gly	Leu	Gln	
	1680					1685					1690					
CCC	ACA	GTG	GAG	TAT	GTG	GTT	AGT	GTC	TAT	GCT	CAG	AAT	CCA	AGC	GGA	5135
Pro	Thr	Val	Glu	Tyr	Val	Val	Ser	Val	Tyr	Ala	Gln	Asn	Pro	Ser	Gly	
1695					1700				1705						1710	
GAG	AGT	CAG	CCT	CTG	GTT	CAG	ACT	GCA	GTA	ACC	AAC	ATT	GAT	CGC	CCT	5183
Glu	Ser	Gln	Pro	Leu	Val	Gln	Thr	Ala	Val	Thr	Asn	Ile	Asp	Arg	Pro	
			1715					1720						1725		

AAA GGA CTG GCA TTC ACT GAT GTG GAT GTC GAT TCC ATC AAA ATT GCT Lys Gly Leu Ala Phe Thr Asp Val Asp Val Asp Ser Ile Lys Ile Ala 1730 1735 1740	5231
TGG GAA AGC CCA CAG GGG CAA GTT TCC AGG TAC AGG GTG ACC TAC TCG Trp Glu Ser Pro Gln Gly Gln Val Ser Arg Tyr Arg Val Thr Tyr Ser 1745 1750 1755	5279
AGC CCT GAG GAT GGA ATC CAT GAG CTA TTC CCT GCA CCT GAT GGT GAA Ser Pro Glu Asp Gly Ile His Glu Leu Phe Pro Ala Pro Asp Gly Glu 1760 1765 1770	5327
GAA GAC ACT GCA GAG CTG CAA GGC CTC AGA CCG GGT TCT GAG TAC ACA Glu Asp Thr Ala Glu Leu Gln Gly Leu Arg Pro Gly Ser Glu Tyr Thr 1775 1780 1785 1790	5375
GTC AGT GTG GTT GCC TTG CAC GAT GAT ATG GAG AGC CAG CCC CTG ATT Val Ser Val Val Ala Leu His Asp Asp Met Glu Ser Gln Pro Leu Ile 1795 1800 1805	5423
GGA ACC CAG TCC ACA GCT ATT CCT GCA CCA ACT GAC CTG AAG TTC ACT Gly Thr Gln Ser Thr Ala Ile Pro Ala Pro Thr Asp Leu Lys Phe Thr 1810 1815 1820	5471
CAG GTC ACA CCC ACA AGC CTG AGC GCC CAG TGG ACA CCA CCC AAT GTT Gln Val Thr Pro Thr Ser Leu Ser Ala Gln Trp Thr Pro Pro Asn Val 1825 1830 1835	5519
CAG CTC ACT GGA TAT CGA GTG CGG GTG ACC CCC AAG GAG AAG ACC GGA Gln Leu Thr Gly Tyr Arg Val Arg Val Thr Pro Lys Glu Lys Thr Gly 1840 1845 1850	5567
CCA ATG AAA GAA ATC AAC CTT GCT CCT GAC AGC TCA TCC GTG GTT GTA Pro Met Lys Glu Ile Asn Leu Ala Pro Asp Ser Ser Ser Val Val Val 1855 1860 1865 1870	5615
TCA GGA CTT ATG GTG GCC ACC AAA TAT GAA GTG AGT GTC TAT GCT CTT Ser Gly Leu Met Val Ala Thr Lys Tyr Glu Val Ser Val Tyr Ala Leu 1875 1880 1885	5663
AAG GAC ACT TTG ACA AGC AGA CCA GCT CAG GGT GTT GTC ACC ACT CTG Lys Asp Thr Leu Thr Ser Arg Pro Ala Gln Gly Val Val Thr Thr Leu 1890 1895 1900	5711
GAG AAT GTC AGC CCA CCA AGA AGG GCT CGT GTG ACA GAT GCT ACT GAG Glu Asn Val Ser Pro Pro Arg Arg Ala Arg Val Thr Asp Ala Thr Glu 1905 1910 1915	5759
ACC ACC ATC ACC ATT AGC TGG AGA ACC AAG ACT GAG ACG ATC ACT GGC Thr Thr Ile Thr Ile Ser Trp Arg Thr Lys Thr Glu Thr Ile Thr Gly 1920 1925 1930	5807

TTC CAA GTT GAT GCC GTT CCA GCC AAT GGC CAG ACT CCA ATC CAG AGA Phe Gln Val Asp Ala Val Pro Ala Asn Gly Gln Thr Pro Ile Gln Arg 1935 1940 1945 1950	5855
ACC ATC AAG CCA GAT GTC AGA AGC TAC ACC ATC ACA GGT TTA CAA CCA Thr Ile Lys Pro Asp Val Arg Ser Tyr Thr Ile Thr Gly Leu Gln Pro 1955 1960 1965	5903
GGC ACT GAC TAC AAG ATC TAC CTG TAC ACC TTG AAT GAC AAT GCT CGG Gly Thr Asp Tyr Lys Ile Tyr Leu Tyr Thr Leu Asn Asp Asn Ala Arg 1970 1975 1980	5951
AGC TCC CCT GTG GTC ATC GAC GCC TCC ACT GCC ATT GAT GCA CCA TCC Ser Ser Pro Val Val Ile Asp Ala Ser Thr Ala Ile Asp Ala Pro Ser 1985 1990 1995	5999
AAC CTG CGT TTC CTG GCC ACC ACA CCC AAT TCC TTG CTG GTA TCA TGG Asn Leu Arg Phe Leu Ala Thr Thr Pro Asn Ser Leu Leu Val Ser Trp 2000 2005 2010	6047
CAG CCG CCA CGT GCC AGG ATT ACC GGC TAC ATC ATC AAG TAT GAG AAG Gln Pro Pro Arg Ala Arg Ile Thr Gly Tyr Ile Ile Lys Tyr Glu Lys 2015 2020 2025 2030	6095
CCT GGG TCT CCT CCC AGA GAA GTG GTC CCT CGG CCC CGC CCT GGT GTC Pro Gly Ser Pro Pro Arg Glu Val Val Pro Arg Pro Arg Pro Gly Val 2035 2040 2045	6143
ACA GAG GCT ACT ATT ACT GGC CTG GAA CCG GGA ACC GAA TAT ACA ATT Thr Glu Ala Thr Ile Thr Gly Leu Glu Pro Gly Thr Glu Tyr Thr Ile 2050 2055 2060	6191
TAT GTC ATT GCC CTG AAG AAT AAT CAG AAG AGC GAG CCC CTG ATT GGA Tyr Val Ile Ala Leu Lys Asn Asn Gln Lys Ser Glu Pro Leu Ile Gly 2065 2070 2075	6239
AGG AAA AAG ACA GAC GAG CTT CCC CAA CTG GTA ACC CTT CCA CAC CCC Arg Lys Lys Thr Asp Glu Leu Pro Gln Leu Val Thr Leu Pro His Pro 2080 2085 2090	6287
AAT CTT CAT GGA CCA GAG ATC TTG GAT GTT CCT TCC ACA GTT CAA AAG Asn Leu His Gly Pro Glu Ile Leu Asp Val Pro Ser Thr Val Gln Lys 2095 2100 2105 2110	6335
ACC CCT TTC GTC ACC CAC CCT GGG TAT GAC ACT GGA AAT GGT ATT CAG Thr Pro Phe Val Thr His Pro Gly Tyr Asp Thr Gly Asn Gly Ile Gln 2115 2120 2125	6383
CTT CCT GGC ACT TCT GGT CAG CAA CCC AGT GTT GGG CAA CAA ATG ATC Leu Pro Gly Thr Ser Gly Gln Gln Pro Ser Val Gly Gln Gln Met Ile 2130 2135 2140	6431
TTT GAG GAA CAT GGT TTT AGG CGG ACC ACA CCG CCC ACA ACG GCC ACC Phe Glu Glu His Gly Phe Arg Arg Thr Thr Pro Pro Thr Thr Ala Thr 2145 2150 2155	6479

CCC ATA AGG CAT AGG CCA AGA CCA TAC CCG CCG AAT GTA GGA CAA GAA Pro Ile Arg His Arg Pro Arg Pro Tyr Pro Pro Asn Val Gly Gln Glu 2160 2165 2170	6527
GCT CTC TCT CAG ACA ACC ATC TCA TGG GCC CCA TTC CAG GAC ACT TCT Ala Leu Ser Gln Thr Thr Ile Ser Trp Ala Pro Phe Gln Asp Thr Ser 2175 2180 2185 2190	6575
GAG TAC ATC ATT TCA TGT CAT CCT GTT GGC ACT GAT GAA GAA CCC TTA Glu Tyr Ile Ile Ser Cys His Pro Val Gly Thr Asp Glu Glu Pro Leu 2195 2200 2205	6623
CAG TTC AGG GTT CCT GGA ACT TCT ACC AGT GCC ACT CTG ACA GGC CTC Gln Phe Arg Val Pro Gly Thr Ser Thr Ser Ala Thr Leu Thr Gly Leu 2210 2215 2220	6671
ACC AGA GGT GCC ACC TAC AAC ATC ATA GTG GAG GCA CTG AAA GAC CAG Thr Arg Gly Ala Thr Tyr Asn Ile Ile Val Glu Ala Leu Lys Asp Gln 2225 2230 2235	6719
CAG AGG CAT AAG GTT CGG GAA GAG GTT GTT ACC GTG GGC AAC TCT GTC Gln Arg His Lys Val Arg Glu Glu Val Val Thr Val Gly Asn Ser Val 2240 2245 2250	6767
AAC GAA GGC TTG AAC CAA CCT ACG GAT GAC TCG TGC TTT GAC CCC TAC Asn Glu Gly Leu Asn Gln Pro Thr Asp Asp Ser Cys Phe Asp Pro Tyr 2255 2260 2265 2270	6815
ACA GTT TCC CAT TAT GCC GTT GGA GAT GAG TGG GAA CGA ATG TCT GAA Thr Val Ser His Tyr Ala Val Gly Asp Glu Trp Glu Arg Met Ser Glu 2275 2280 2285	6863
TCA GGC TTT AAA CTG TTG TGC CAG TGC TTA GGC TTT GGA AGT GGT CAT Ser Gly Phe Lys Leu Leu Cys Gln Cys Leu Gly Phe Gly Ser Gly His 2290 2295 2300	6911
TTC AGA TGT GAT TCA TCT AGA TGG TGC CAT GAC AAT GGT GTG AAC TAC Phe Arg Cys Asp Ser Ser Arg Trp Cys His Asp Asn Gly Val Asn Tyr 2305 2310 2315	6959
AAG ATT GGA GAG AAG TGG GAC CGT CAG GGA GAA AAT GGC CAG ATG ATG Lys Ile Gly Glu Lys Trp Asp Arg Gln Gly Glu Asn Gly Gln Met Met 2320 2325 2330	7007
AGC TGC ACA TGT CTT GGG AAC GGA AAA GGA GAA TTC AAG TGT GAC CCT Ser Cys Thr Cys Leu Gly Asn Gly Lys Gly Glu Phe Lys Cys Asp Pro 2335 2340 2345 2350	7055
CAT GAG GCA ACG TGT TAC GAT GAT GGG AAG ACA TAC CAC GTA GGA GAA His Glu Ala Thr Cys Tyr Asp Asp Gly Lys Thr Tyr His Val Gly Glu 2355 2360 2365	7103

48

CAG TGG CAG AAG GAA TAT CTC GGT GCC ATT TGC TCC TGC ACA TGC TTT	7151
Gln Trp Gln Lys Glu Tyr Leu Gly Ala Ile Cys Ser Cys Thr Cys Phe	
2370 2375 2380	
GGA GGC CAG CGG GGC TGG CGC TGT GAC AAC TGC CGC AGA CCT GGG GGT	7199
Gly Gly Gln Arg Gly Trp Arg Cys Asp Asn Cys Arg Arg Pro Gly Gly	
2385 2390 2395	
GAA CCC AGT CCC GAA GGC ACT ACT GGC CAG TCC TAC AAC CAG TAT TCT	7247
Glu Pro Ser Pro Glu Gly Thr Thr Gly Gln Ser Tyr Asn Gln Tyr Ser	
2400 2405 2410	
CAG AGA TAC CAT CAG AGA ACA AAC ACT AAT GTT AAT TGC CCA ATT GAG	7295
Gln Arg Tyr His Gln Arg Thr Asn Thr Asn Val Asn Cys Pro Ile Glu	
2415 2420 2425 2430	
TGC TTC ATG CCT TTA GAT GTA CAG GCT GAC AGA GAA GAT TCC CGA GAG	7343
Cys Phe Met Pro Leu Asp Val Gln Ala Asp Arg Glu Asp Ser Arg Glu	
2435 2440 2445	
TAAATCATCT TTCCAATCCA GAGGAACAAG CATGTCTCTC TGCCAAGATC CATCTAACT	7403
GGAGTGATGT TAGCAGACCC AGCTTAGAGT TCTTCTTTCT TTCTTAAGCC CTTTGCTCTG	7463
GAGGAAGTTC TCCAGCTTCA GCTCAACTCA CAGCTTCTCC AAGCATCACC CTGGGAGTTT	7523
CCTGAGGGTT TTCTCATAAA TGAGGGCTGC ACATTGCCTG TTCTGCTTCG AAGTATTCAA	7583
TACCGCTCAG TATTTTAAAT GAAGTGATTC TAAGATTTGG TTTGGGATCA ATAGGAAAGC	7643
ATATGCAGCC AACCAAGATG CAAATGTTTT GAAATGATAT GACCAAAATT TTAAGTAGGA	7703
AAGTCACCCA AACACTTCTG CTTTCACTTA AGTGTCTGGC CCGCAATACT GTAGGAACAA	7763
GCATGATCTT GTTACTGTGA TATTTTAAAT ATCCACAGTA	7803

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2446 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met	Leu	Arg	Gly	Pro	Gly	Pro	Gly	Leu	Leu	Leu	Leu	Ala	Val	Leu	Cys
1				5				10						15	
Leu	Gly	Thr	Ala	Val	Pro	Ser	Thr	Gly	Ala	Ser	Lys	Ser	Lys	Arg	Gln
			20					25					30		

Ala Gln Gln Met Val Gln Pro Gln Ser Pro Val Ala Val Ser Gln Ser
 35 40 45
 Lys Pro Gly Cys Tyr Asp Asn Gly Lys His Tyr Gln Ile Asn Gln Gln
 50 55 60
 Trp Glu Arg Thr Tyr Leu Gly Asn Val Leu Val Cys Thr Cys Tyr Gly
 65 70 75 80
 Gly Ser Arg Gly Phe Asn Cys Glu Ser Lys Pro Glu Ala Glu Glu Thr
 85 90 95
 Cys Phe Asp Lys Tyr Thr Gly Asn Thr Tyr Arg Val Gly Asp Thr Tyr
 100 105 110
 Glu Arg Pro Lys Asp Ser Met Ile Trp Asp Cys Thr Cys Ile Gly Ala
 115 120 125
 Gly Arg Gly Arg Ile Ser Cys Thr Ile Ala Asn Arg Cys His Glu Gly
 130 135 140
 Gly Gln Ser Tyr Lys Ile Gly Asp Thr Trp Arg Arg Pro His Glu Thr
 145 150 155 160
 Gly Gly Tyr Met Leu Glu Cys Val Cys Leu Gly Asn Gly Lys Gly Glu
 165 170 175
 Trp Thr Cys Lys Pro Ile Ala Glu Lys Cys Phe Asp His Ala Ala Gly
 180 185 190
 Thr Ser Tyr Val Val Gly Glu Thr Trp Glu Lys Pro Tyr Gln Gly Trp
 195 200 205
 Met Met Val Asp Cys Thr Cys Leu Gly Glu Gly Ser Gly Arg Ile Thr
 210 215 220
 Cys Thr Ser Arg Asn Arg Cys Asn Asp Gln Asp Thr Arg Thr Ser Tyr
 225 230 235 240
 Arg Ile Gly Asp Thr Trp Ser Lys Lys Asp Asn Arg Gly Asn Leu Leu
 245 250 255
 Gln Cys Ile Cys Thr Gly Asn Gly Arg Gly Glu Trp Lys Cys Glu Arg
 260 265 270
 His Thr Ser Val Gln Thr Thr Ser Ser Gly Ser Gly Pro Phe Thr Asp
 275 280 285
 Val Arg Ala Ala Val Tyr Gln Pro Gln Pro His Pro Gln Pro Pro Pro
 290 295 300
 Tyr Gly His Cys Val Thr Asp Ser Gly Val Val Tyr Ser Val Gly Met
 305 310 315 320

Gln Trp Leu Lys Thr Gln Gly Asn Lys Gln Met Leu Cys Thr Cys Leu
 325 330 335
 Gly Asn Gly Val Ser Cys Gln Glu Thr Ala Val Thr Gln Thr Tyr Gly
 340 345 350
 Gly Asn Leu Asn Gly Glu Pro Cys Val Leu Pro Phe Thr Tyr Asn Gly
 355 360 365
 Arg Thr Phe Tyr Ser Cys Thr Thr Glu Gly Arg Gln Asp Gly His Leu
 370 375 380
 Trp Cys Ser Thr Thr Ser Asn Tyr Glu Gln Asp Gln Lys Tyr Ser Phe
 385 390 395 400
 Cys Thr Asp His Thr Val Leu Val Gln Thr Gln Gly Gly Asn Ser Asn
 405 410 415
 Gly Ala Leu Cys His Phe Pro Phe Leu Tyr Asn Asn His Asn Tyr Thr
 420 425 430
 Asp Cys Thr Ser Glu Gly Arg Arg Asp Asn Met Lys Trp Cys Gly Thr
 435 440 445
 Thr Gln Asn Tyr Asp Ala Asp Gln Lys Phe Gly Phe Cys Pro Met Ala
 450 455 460
 Ala His Glu Glu Ile Cys Thr Thr Asn Glu Gly Val Met Tyr Arg Ile
 465 470 475 480
 Gly Asp Gln Trp Asp Lys Gln His Asp Met Gly His Met Met Arg Cys
 485 490 495
 Thr Cys Val Gly Asn Gly Arg Gly Glu Trp Thr Cys Ile Ala Tyr Ser
 500 505 510
 Gln Leu Arg Asp Gln Cys Ile Val Asp Asp Ile Thr Tyr Asn Val Asn
 515 520 525
 Asp Thr Phe His Lys Arg His Glu Glu Gly His Met Leu Asn Cys Thr
 530 535 540
 Cys Phe Gly Gln Gly Arg Gly Arg Trp Lys Cys Asp Pro Val Asp Gln
 545 550 555 560
 Cys Gln Asp Ser Glu Thr Gly Thr Phe Tyr Gln Ile Gly Asp Ser Trp
 565 570 575
 Glu Lys Tyr Val His Gly Val Arg Tyr Gln Cys Tyr Cys Tyr Gly Arg
 580 585 590
 Gly Ile Gly Glu Trp His Cys Gln Pro Leu Gln Thr Tyr Pro Ser Ser
 595 600 605

Ser Gly Pro Val Glu Val Phe Ile Thr Glu Thr Pro Ser Gln Pro Asn
 610 615 620
 Ser His Pro Ile Gln Trp Asn Ala Pro Gln Pro Ser His Ile Ser Lys
 625 630 635 640
 Tyr Ile Leu Arg Trp Arg Pro Lys Asn Ser Val Gly Arg Trp Lys Glu
 645 650 655
 Ala Thr Ile Pro Gly His Leu Asn Ser Tyr Thr Ile Lys Gly Leu Lys
 660 665 670
 Pro Gly Val Val Tyr Glu Gly Gln Leu Ile Ser Ile Gln Gln Tyr Gly
 675 680 685
 His Gln Glu Val Thr Arg Phe Asp Phe Thr Thr Thr Ser Thr Ser Thr
 690 695 700
 Pro Val Thr Ser Asn Thr Val Thr Gly Glu Thr Thr Pro Phe Ser Pro
 705 710 715 720
 Leu Val Ala Thr Ser Glu Ser Val Thr Glu Ile Thr Ala Ser Ser Phe
 725 730 735
 Val Val Ser Trp Val Ser Ala Ser Asp Thr Val Ser Gly Phe Arg Val
 740 745 750
 Glu Tyr Glu Leu Ser Glu Glu Gly Asp Glu Pro Gln Tyr Leu Asp Leu
 755 760 765
 Pro Ser Thr Ala Thr Ser Val Asn Ile Pro Asp Leu Leu Pro Gly Arg
 770 775 780
 Lys Tyr Ile Val Asn Val Tyr Gln Ile Ser Glu Asp Gly Glu Gln Ser
 785 790 795 800
 Leu Ile Leu Ser Thr Ser Gln Thr Thr Ala Pro Asp Ala Pro Pro Asp
 805 810 815
 Pro Thr Val Asp Gln Val Asp Asp Thr Ser Ile Val Val Arg Trp Ser
 820 825 830
 Arg Pro Gln Ala Pro Ile Thr Gly Tyr Arg Ile Val Tyr Ser Pro Ser
 835 840 845
 Val Glu Gly Ser Ser Thr Glu Leu Asn Leu Pro Glu Thr Ala Asn Ser
 850 855 860
 Val Thr Leu Ser Asp Leu Gln Pro Gly Val Gln Tyr Asn Ile Thr Ile
 865 870 875 880
 Tyr Ala Val Glu Glu Asn Gln Glu Ser Thr Pro Val Val Ile Gln Gln
 885 890 895

Glu Thr Thr Gly Thr Pro Arg Ser Asp Thr Val Pro Ser Pro Arg Asp
 900 905 910
 Leu Gln Phe Val Glu Val Thr Asp Val Lys Val Thr Ile Met Trp Thr
 915 920 925
 Pro Pro Glu Ser Ala Val Thr Gly Tyr Arg Val Asp Val Ile Pro Val
 930 935 940
 Asn Leu Pro Gly Glu His Gly Gln Arg Leu Pro Ile Ser Arg Asn Thr
 945 950 955 960
 Phe Ala Glu Val Thr Gly Leu Ser Pro Gly Val Thr Tyr Tyr Phe Lys
 965 970 975
 Val Phe Ala Val Ser His Gly Arg Glu Ser Lys Pro Leu Thr Ala Gln
 980 985 990
 Gln Thr Thr Lys Leu Asp Ala Pro Thr Asn Leu Gln Phe Val Asn Glu
 995 1000 1005
 Thr Asp Ser Thr Val Leu Val Arg Trp Thr Pro Pro Arg Ala Gln Ile
 1010 1015 1020
 Thr Gly Tyr Arg Leu Thr Val Gly Leu Thr Arg Arg Gly Gln Pro Arg
 1025 1030 1035 1040
 Gln Tyr Asn Val Gly Pro Ser Val Ser Lys Tyr Pro Leu Arg Asn Leu
 1045 1050 1055
 Gln Pro Ala Ser Glu Tyr Thr Val Ser Leu Val Ala Ile Lys Gly Asn
 1060 1065 1070
 Gln Glu Ser Pro Lys Ala Thr Gly Val Phe Thr Thr Leu Gln Pro Gly
 1075 1080 1085
 Ser Ser Ile Pro Pro Tyr Asn Thr Glu Val Thr Glu Thr Thr Ile Val
 1090 1095 1100
 Ile Thr Trp Thr Pro Ala Pro Arg Ile Gly Phe Lys Leu Gly Val Arg
 1105 1110 1115 1120
 Pro Ser Gln Gly Gly Glu Ala Pro Arg Glu Val Thr Ser Asp Ser Gly
 1125 1130 1135
 Ser Ile Val Val Ser Gly Leu Thr Pro Gly Val Glu Tyr Val Tyr Thr
 1140 1145 1150
 Ile Gln Val Leu Arg Asp Gly Gln Glu Arg Asp Ala Pro Ile Val Asn
 1155 1160 1165
 Lys Val Val Thr Pro Leu Ser Pro Pro Thr Asn Leu His Leu Glu Ala
 1170 1175 1180

Asn Pro Asp Thr Gly Val Leu Thr Val Ser Trp Glu Arg Ser Thr Thr
 1185 1190 1195 1200
 Pro Asp Ile Thr Gly Tyr Arg Ile Thr Thr Thr Pro Thr Asn Gly Gln
 1205 1210 1215
 Gln Gly Asn Ser Leu Glu Glu Val Val His Ala Asp Gln Ser Ser Cys
 1220 1225 1230
 Thr Phe Asp Asn Leu Ser Pro Gly Leu Glu Tyr Asn Val Ser Val Tyr
 1235 1240 1245
 Thr Val Lys Asp Asp Lys Glu Ser Val Pro Ile Ser Asp Thr Ile Ile
 1250 1255 1260
 Pro Glu Val Pro Gln Leu Thr Asp Leu Ser Phe Val Asp Ile Thr Asp
 1265 1270 1275 1280
 Ser Ser Ile Gly Leu Arg Trp Thr Pro Leu Asn Ser Ser Thr Ile Ile
 1285 1290 1295
 Gly Tyr Arg Ile Thr Val Val Ala Ala Gly Glu Gly Ile Pro Ile Phe
 1300 1305 1310
 Glu Asp Phe Val Tyr Ser Ser Val Gly Tyr Tyr Thr Val Thr Gly Leu
 1315 1320 1325
 Glu Pro Gly Ile Asp Tyr Asp Ile Ser Val Ile Thr Leu Ile Asn Gly
 1330 1335 1340
 Gly Glu Ser Ala Pro Thr Thr Leu Thr Gln Gln Thr Ala Val Pro Pro
 1345 1350 1355 1360
 Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met Arg Val
 1365 1370 1375
 Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu Thr Asn Phe Leu Val Arg
 1380 1385 1390
 Tyr Ser Pro Val Lys Asn Glu Glu Asp Val Ala Glu Leu Ser Ile Ser
 1395 1400 1405
 Pro Ser Asp Asn Ala Val Val Leu Thr Asn Leu Leu Pro Gly Thr Glu
 1410 1415 1420
 Tyr Val Val Ser Val Ser Ser Val Tyr Glu Gln His Glu Ser Thr Pro
 1425 1430 1435 1440
 Leu Arg Gly Arg Gln Lys Thr Gly Leu Asp Ser Pro Thr Gly Ile Asp
 1445 1450 1455
 Phe Ser Asp Ile Thr Ala Asn Ser Phe Thr Val His Trp Ile Ala Pro
 1460 1465 1470

Arg Ala Thr Ile Thr Gly Tyr Arg Ile Arg His His Pro Glu His Phe
 1475 1480 1485
 Ser Gly Arg Pro Arg Glu Asp Arg Val Pro His Ser Arg Asn Ser Ile
 1490 1495 1500
 Thr Leu Thr Asn Leu Thr Pro Gly Thr Glu Tyr Val Val Ser Ile Val
 1505 1510 1515 1520
 Ala Leu Asn Gly Arg Glu Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser
 1525 1530 1535
 Thr Val Ser Asp Val Pro Arg Asp Leu Glu Val Val Ala Ala Thr Pro
 1540 1545 1550
 Thr Ser Leu Leu Ile Ser Trp Asp Ala Pro Ala Val Thr Val Arg Tyr
 1555 1560 1565
 Tyr Arg Ile Thr Tyr Gly Glu Thr Gly Gly Asn Ser Pro Val Gln Glu
 1570 1575 1580
 Phe Thr Val Pro Gly Ser Lys Ser Thr Ala Thr Ile Ser Gly Leu Lys
 1585 1590 1595 1600
 Pro Gly Val Asp Tyr Thr Ile Thr Val Tyr Ala Val Thr Gly Arg Gly
 1605 1610 1615
 Asp Ser Pro Ala Ser Ser Lys Pro Ile Ser Ile Asn Tyr Arg Thr Glu
 1620 1625 1630
 Ile Asp Lys Pro Ser Gln Met Gln Val Thr Asp Val Gln Asp Asn Ser
 1635 1640 1645
 Ile Ser Val Lys Trp Leu Pro Ser Ser Ser Pro Val Thr Gly Tyr Arg
 1650 1655 1660
 Val Thr Thr Thr Pro Lys Asn Gly Pro Gly Pro Thr Lys Thr Lys Thr
 1665 1670 1675 1680
 Ala Gly Pro Asp Gln Thr Glu Met Thr Ile Glu Gly Leu Gln Pro Thr
 1685 1690 1695
 Val Glu Tyr Val Val Ser Val Tyr Ala Gln Asn Pro Ser Gly Glu Ser
 1700 1705 1710
 Gln Pro Leu Val Gln Thr Ala Val Thr Asn Ile Asp Arg Pro Lys Gly
 1715 1720 1725
 Leu Ala Phe Thr Asp Val Asp Val Asp Ser Ile Lys Ile Ala Trp Glu
 1730 1735 1740
 Ser Pro Gln Gly Gln Val Ser Arg Tyr Arg Val Thr Tyr Ser Ser Pro
 1745 1750 1755 1760

Glu Asp Gly Ile His Glu Leu Phe Pro Ala Pro Asp Gly Glu Glu Asp
 1765 1770 1775
 Thr Ala Glu Leu Gln Gly Leu Arg Pro Gly Ser Glu Tyr Thr Val Ser
 1780 1785 1790
 Val Val Ala Leu His Asp Asp Met Glu Ser Gln Pro Leu Ile Gly Thr
 1795 1800 1805
 Gln Ser Thr Ala Ile Pro Ala Pro Thr Asp Leu Lys Phe Thr Gln Val
 1810 1815 1820
 Thr Pro Thr Ser Leu Ser Ala Gln Trp Thr Pro Pro Asn Val Gln Leu
 1825 1830 1835 1840
 Thr Gly Tyr Arg Val Arg Val Thr Pro Lys Glu Lys Thr Gly Pro Met
 1845 1850 1855
 Lys Glu Ile Asn Leu Ala Pro Asp Ser Ser Ser Val Val Val Ser Gly
 1860 1865 1870
 Leu Met Val Ala Thr Lys Tyr Glu Val Ser Val Tyr Ala Leu Lys Asp
 1875 1880 1885
 Thr Leu Thr Ser Arg Pro Ala Gln Gly Val Val Thr Thr Leu Glu Asn
 1890 1895 1900
 Val Ser Pro Pro Arg Arg Ala Arg Val Thr Asp Ala Thr Glu Thr Thr
 1905 1910 1915 1920
 Ile Thr Ile Ser Trp Arg Thr Lys Thr Glu Thr Ile Thr Gly Phe Gln
 1925 1930 1935
 Val Asp Ala Val Pro Ala Asn Gly Gln Thr Pro Ile Gln Arg Thr Ile
 1940 1945 1950
 Lys Pro Asp Val Arg Ser Tyr Thr Ile Thr Gly Leu Gln Pro Gly Thr
 1955 1960 1965
 Asp Tyr Lys Ile Tyr Leu Tyr Thr Leu Asn Asp Asn Ala Arg Ser Ser
 1970 1975 1980
 Pro Val Val Ile Asp Ala Ser Thr Ala Ile Asp Ala Pro Ser Asn Leu
 1985 1990 1995 2000
 Arg Phe Leu Ala Thr Thr Pro Asn Ser Leu Leu Val Ser Trp Gln Pro
 2005 2010 2015
 Pro Arg Ala Arg Ile Thr Gly Tyr Ile Ile Lys Tyr Glu Lys Pro Gly
 2020 2025 2030
 Ser Pro Pro Arg Glu Val Val Pro Arg Pro Arg Pro Gly Val Thr Glu
 2035 2040 2045

Ala Thr Ile Thr Gly Leu Glu Pro Gly Thr Glu Tyr Thr Ile Tyr Val
 2050 2055 2060
 Ile Ala Leu Lys Asn Asn Gln Lys Ser Glu Pro Leu Ile Gly Arg Lys
 2065 2070 2075 2080
 Lys Thr Asp Glu Leu Pro Gln Leu Val Thr Leu Pro His Pro Asn Leu
 2085 2090 2095
 His Gly Pro Glu Ile Leu Asp Val Pro Ser Thr Val Gln Lys Thr Pro
 2100 2105 2110
 Phe Val Thr His Pro Gly Tyr Asp Thr Gly Asn Gly Ile Gln Leu Pro
 2115 2120 2125
 Gly Thr Ser Gly Gln Gln Pro Ser Val Gly Gln Gln Met Ile Phe Glu
 2130 2135 2140
 Glu His Gly Phe Arg Arg Thr Thr Pro Pro Thr Thr Ala Thr Pro Ile
 2145 2150 2155 2160
 Arg His Arg Pro Arg Pro Tyr Pro Pro Asn Val Gly Gln Glu Ala Leu
 2165 2170 2175
 Ser Gln Thr Thr Ile Ser Trp Ala Pro Phe Gln Asp Thr Ser Glu Tyr
 2180 2185 2190
 Ile Ile Ser Cys His Pro Val Gly Thr Asp Glu Glu Pro Leu Gln Phe
 2195 2200 2205
 Arg Val Pro Gly Thr Ser Thr Ser Ala Thr Leu Thr Gly Leu Thr Arg
 2210 2215 2220
 Gly Ala Thr Tyr Asn Ile Ile Val Glu Ala Leu Lys Asp Gln Gln Arg
 2225 2230 2235 2240
 His Lys Val Arg Glu Glu Val Val Thr Val Gly Asn Ser Val Asn Glu
 2245 2250 2255
 Gly Leu Asn Gln Pro Thr Asp Asp Ser Cys Phe Asp Pro Tyr Thr Val
 2260 2265 2270
 Ser His Tyr Ala Val Gly Asp Glu Trp Glu Arg Met Ser Glu Ser Gly
 2275 2280 2285
 Phe Lys Leu Leu Cys Gln Cys Leu Gly Phe Gly Ser Gly His Phe Arg
 2290 2295 2300
 Cys Asp Ser Ser Arg Trp Cys His Asp Asn Gly Val Asn Tyr Lys Ile
 2305 2310 2315 2320
 Gly Glu Lys Trp Asp Arg Gln Gly Glu Asn Gly Gln Met Met Ser Cys
 2325 2330 2335

57

Thr Cys Leu Gly Asn Gly Lys Gly Glu Phe Lys Cys Asp Pro His Glu
 2340 2345 2350
 Ala Thr Cys Tyr Asp Asp Gly Lys Thr Tyr His Val Gly Glu Gln Trp
 2355 2360 2365
 Gln Lys Glu Tyr Leu Gly Ala Ile Cys Ser Cys Thr Cys Phe Gly Gly
 2370 2375 2380
 Gln Arg Gly Trp Arg Cys Asp Asn Cys Arg Arg Pro Gly Gly Glu Pro
 2385 2390 2395 2400
 Ser Pro Glu Gly Thr Thr Gly Gln Ser Tyr Asn Gln Tyr Ser Gln Arg
 2405 2410 2415
 Tyr His Gln Arg Thr Asn Thr Asn Val Asn Cys Pro Ile Glu Cys Phe
 2420 2425 2430
 Met Pro Leu Asp Val Gln Ala Asp Arg Glu Asp Ser Arg Glu
 2435 2440 2445

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2179 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 31..1962

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GTCTAGGAGC CAGCCCCACC CTTAGAAAAG ATG TTT TCC ATG AGG ATC GTC TGC	54
Met Phe Ser Met Arg Ile Val Cys	
1 5	
CTA GTT CTA AGT GTG GTG GGC ACA GCA TGG ACT GCA GAT AGT GGT GAA	102
Leu Val Leu Ser Val Val Gly Thr Ala Trp Thr Ala Asp Ser Gly Glu	
10 15 20	
GGT GAC TTT CTA GCT GAA GGA GGA GGC GTG CGT GGC CCA AGG GTT GTG	150
Gly Asp Phe Leu Ala Glu Gly Gly Gly Val Arg Gly Pro Arg Val Val	
25 30 35 40	
GAA AGA CAT CAA TCT GCC TGC AAA GAT TCA GAC TGG CCC TTC TGC TCT	198
Glu Arg His Gln Ser Ala Cys Lys Asp Ser Asp Trp Pro Phe Cys Ser	
45 50 55	

GAT Asp	GAA Glu	GAC Asp	TGG Trp 60	AAC Asn	TAC Tyr	AAA Lys	TGC Cys	CCT Pro 65	TCT Ser	GGC Gly	TGC Cys	AGG Arg	ATG Met 70	AAA Lys	GGG Gly	246
TTG Leu	ATT Ile	GAT Asp 75	GAA Glu	GTC Val	AAT Asn	CAA Gln	GAT Asp 80	TTT Phe	ACA Thr	AAC Asn	AGA Arg	ATA Ile 85	AAT Asn	AAG Lys	CTC Leu	294
AAA Lys	AAT Asn 90	TCA Ser	CTA Leu	TTT Phe	GAA Glu	TAT Tyr 95	CAG Gln	AAG Lys	AAC Asn	AAT Asn	AAG Lys 100	GAT Asp	TCT Ser	CAT His	TCG Ser	342
TTG Leu 105	ACC Thr	ACT Thr	AAT Asn	ATA Ile	ATG Met 110	GAA Glu	ATT Ile	TTG Leu	AGA Arg	GGC Gly 115	GAT Asp	TTT Phe	TCC Ser	TCA Ser	GCC Ala 120	390
AAT Asn	AAC Asn	CGT Arg	GAT Asp	AAT Asn 125	ACC Thr	TAC Tyr	AAC Asn	CGA Arg	GTG Val 130	TCA Ser	GAG Glu	GAT Asp	CTG Leu 135	AGA Arg	AGC Ser	438
AGA Arg	ATT Ile	GAA Glu	GTC Val 140	CTG Leu	AAG Lys	CGC Arg	AAA Lys	GTC Val 145	ATA Ile	GAA Glu	AAA Lys	GTA Val 150	CAG Gln	CAT His	ATC Ile	486
CAG Gln	CTT Leu 155	CTG Leu	CAG Gln	AAA Lys	AAT Asn	GTT Val	AGA Arg 160	GCT Ala	CAG Gln	TTG Leu	GTT Val	GAT Asp 165	ATG Met	AAA Lys	CGA Arg	534
CTG Leu 170	GAG Glu	GTG Val	GAC Asp	ATT Ile	GAT Asp	ATT Ile 175	AAG Lys	ATC Ile	CGA Arg	TCT Ser	TGT Cys 180	CGA Arg	GGG Gly	TCA Ser	TGG Trp	582
AGT Ser 185	AGG Arg	GCT Ala	TTA Leu	GCT Ala	CGT Arg 190	GAA Glu	GTA Val	GAT Asp	CTG Leu	AAG Lys 195	GAC Asp	TAT Tyr	GAA Glu	GAT Asp	CAG Gln 200	630
CAG Gln	AAG Lys	CAA Gln	CTT Leu	GAA Glu 205	CAG Gln	GTC Val	ATT Ile	GCC Ala 210	AAA Lys	GAC Asp	TTA Leu	CTT Leu	CCC Pro 215	TCT Ser	AGA Arg	678
GAT Asp	AGG Arg	CAA Gln	CAC His 220	TTA Leu	CCA Pro	CTG Leu	ATA Ile	AAA Lys 225	ATG Met	AAA Lys	CCA Pro	GTT Val 230	CCA Pro	GAC Asp	TTG Leu	726
GTT Val	CCC Pro	GGA Gly 235	AAT Asn	TTT Phe	AAG Lys	AGC Ser	CAG Gln 240	CTT Leu	CAG Gln	AAG Lys	GTA Val	CCC Pro 245	CCA Pro	GAG Glu	TGG Trp	774
AAG Lys	GCA Ala 250	TTA Leu	ACA Thr	GAC Asp	ATG Met	CCG Pro 255	CAG Gln	ATG Met	AGA Arg	ATG Met	GAG Glu 260	TTA Leu	GAG Glu	AGA Arg	CCT Pro	822
GGT Gly 265	GGA Gly	AAT Asn	GAG Glu	ATT Ile	ACT Thr 270	CGA Arg	GGA Gly	GGC Gly	TCC Ser	ACC Thr 275	TCT Ser	TAT Tyr	GGA Gly	ACC Thr	GGA Gly 280	870

TCA	GAG	ACG	GAA	AGC	CCC	AGG	AAC	CCT	AGC	AGT	GCT	GGA	AGC	TGG	AAC	918
Ser	Glu	Thr	Glu	Ser	Pro	Arg	Asn	Pro	Ser	Ser	Ala	Gly	Ser	Trp	Asn	
				285					290					295		
TCT	GGG	AGC	TCT	GGA	CCT	GGA	AGT	ACT	GGA	AAC	CGA	AAC	CCT	GGG	AGC	966
Ser	Gly	Ser	Ser	Gly	Pro	Gly	Ser	Thr	Gly	Asn	Arg	Asn	Pro	Gly	Ser	
			300					305					310			
TCT	GGG	ACT	GGA	GGG	ACT	GCA	ACC	TGG	AAA	CCT	GGG	AGC	TCT	GGA	CCT	1014
Ser	Gly	Thr	Gly	Gly	Thr	Ala	Thr	Trp	Lys	Pro	Gly	Ser	Ser	Gly	Pro	
		315					320					325				
GGA	AGT	GCT	GGA	AGC	TGG	AAC	TCT	GGG	AGC	TCT	GGA	ACT	GGA	AGT	ACT	1062
Gly	Ser	Ala	Gly	Ser	Trp	Asn	Ser	Gly	Ser	Ser	Gly	Thr	Gly	Ser	Thr	
	330					335					340					
GGA	AAC	CAA	AAC	CCT	GGA	AGT	CCT	AGA	CCT	GGT	AGT	ACC	GGA	ACC	TGG	1110
Gly	Asn	Gln	Asn	Pro	Gly	Ser	Pro	Arg	Pro	Gly	Ser	Thr	Gly	Thr	Trp	
	345				350					355					360	
AAT	CCT	GGC	AGC	TCT	GAA	CGC	GGA	AGT	GCT	GGG	CAC	TGG	ACC	TCT	GAG	1158
Asn	Pro	Gly	Ser	Ser	Glu	Arg	Gly	Ser	Ala	Gly	His	Trp	Thr	Ser	Glu	
			365					370						375		
AGC	TCT	GTA	TCT	GGT	AGT	ACT	GGA	CAA	TGG	CAC	TCT	GAA	TCT	GGA	AGT	1206
Ser	Ser	Val	Ser	Gly	Ser	Thr	Gly	Gln	Trp	His	Ser	Glu	Ser	Gly	Ser	
			380					385					390			
TTT	AGG	CCA	GAT	AGC	CCA	GGC	TCT	GGG	AAC	GCG	AGG	CCT	AAC	AAC	CCA	1254
Phe	Arg	Pro	Asp	Ser	Pro	Gly	Ser	Gly	Asn	Ala	Arg	Pro	Asn	Asn	Pro	
		395					400					405				
GAC	TGG	GGC	ACA	TTT	GAA	GAG	GTG	TCA	GGA	AAT	GTA	AGT	CCA	GGG	ACA	1302
Asp	Trp	Gly	Thr	Phe	Glu	Glu	Val	Ser	Gly	Asn	Val	Ser	Pro	Gly	Thr	
	410					415					420					
AGG	AGA	GAG	TAC	CAC	ACA	GAA	AAA	CTG	GTC	ACT	AAA	GGA	GAT	AAA	GAG	1350
Arg	Arg	Glu	Tyr	His	Thr	Glu	Lys	Leu	Val	Thr	Lys	Gly	Asp	Lys	Glu	
	425				430					435					440	
CTC	AGG	ACT	GGT	AAA	GAG	AAG	GTC	ACC	TCT	GGT	AGC	ACA	ACC	ACC	ACG	1398
Leu	Arg	Thr	Gly	Lys	Glu	Lys	Val	Thr	Ser	Gly	Ser	Thr	Thr	Thr	Thr	
				445					450					455		
CGT	CGT	TCA	TGC	TCT	AAA	ACC	GTT	ACT	AAG	ACT	GTT	ATT	GGT	CCT	GAT	1446
Arg	Arg	Ser	Cys	Ser	Lys	Thr	Val	Thr	Lys	Thr	Val	Ile	Gly	Pro	Asp	
			460					465					470			
GGT	CAC	AAA	GAA	GTT	ACC	AAA	GAA	GTG	GTG	ACC	TCC	GAA	GAT	GGT	TCT	1494
Gly	His	Lys	Glu	Val	Thr	Lys	Glu	Val	Val	Thr	Ser	Glu	Asp	Gly	Ser	
		475					480					485				

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 643 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Phe Ser Met Arg Ile Val Cys Leu Val Leu Ser Val Val Gly Thr
 1 5 10 15
 Ala Trp Thr Ala Asp Ser Gly Glu Gly Asp Phe Leu Ala Glu Gly Gly
 20 25 30
 Gly Val Arg Gly Pro Arg Val Val Glu Arg His Gln Ser Ala Cys Lys
 35 40 45
 Asp Ser Asp Trp Pro Phe Cys Ser Asp Glu Asp Trp Asn Tyr Lys Cys
 50 55 60
 Pro Ser Gly Cys Arg Met Lys Gly Leu Ile Asp Glu Val Asn Gln Asp
 65 70 75 80
 Phe Thr Asn Arg Ile Asn Lys Leu Lys Asn Ser Leu Phe Glu Tyr Gln
 85 90 95
 Lys Asn Asn Lys Asp Ser His Ser Leu Thr Thr Asn Ile Met Glu Ile
 100 105 110
 Leu Arg Gly Asp Phe Ser Ser Ala Asn Asn Arg Asp Asn Thr Tyr Asn
 115 120 125
 Arg Val Ser Glu Asp Leu Arg Ser Arg Ile Glu Val Leu Lys Arg Lys
 130 135 140
 Val Ile Glu Lys Val Gln His Ile Gln Leu Leu Gln Lys Asn Val Arg
 145 150 155 160
 Ala Gln Leu Val Asp Met Lys Arg Leu Glu Val Asp Ile Asp Ile Lys
 165 170 175
 Ile Arg Ser Cys Arg Gly Ser Trp Ser Arg Ala Leu Ala Arg Glu Val
 180 185 190
 Asp Leu Lys Asp Tyr Glu Asp Gln Gln Lys Gln Leu Glu Gln Val Ile
 195 200 205
 Ala Lys Asp Leu Leu Pro Ser Arg Asp Arg Gln His Leu Pro Leu Ile
 210 215 220
 Lys Met Lys Pro Val Pro Asp Leu Val Pro Gly Asn Phe Lys Ser Gln
 225 230 235 240

Leu	Gln	Lys	Val	Pro	Glu	Trp	Lys	Ala	Leu	Thr	Asp	Met	Pro	Gln
				245				250					255	
Met	Arg	Met	Glu	Leu	Glu	Arg	Pro	Gly	Gly	Asn	Glu	Ile	Thr	Arg
			260					265					270	Gly
Gly	Ser	Thr	Ser	Tyr	Gly	Thr	Gly	Ser	Glu	Thr	Glu	Ser	Pro	Arg
			275				280					285		Asn
Pro	Ser	Ser	Ala	Gly	Ser	Trp	Asn	Ser	Gly	Ser	Ser	Gly	Pro	Gly
			290				295				300			Ser
Thr	Gly	Asn	Arg	Asn	Pro	Gly	Ser	Ser	Gly	Thr	Gly	Gly	Thr	Ala
305					310					315				Thr
Trp	Lys	Pro	Gly	Ser	Ser	Gly	Pro	Gly	Ser	Ala	Gly	Ser	Trp	Asn
				325					330					335
Gly	Ser	Ser	Gly	Thr	Gly	Ser	Thr	Gly	Asn	Gln	Asn	Pro	Gly	Ser
			340					345					350	Pro
Arg	Pro	Gly	Ser	Thr	Gly	Thr	Trp	Asn	Pro	Gly	Ser	Ser	Glu	Arg
			355				360					365		Gly
Ser	Ala	Gly	His	Trp	Thr	Ser	Glu	Ser	Ser	Val	Ser	Gly	Ser	Thr
			370				375				380			Gly
Gln	Trp	His	Ser	Glu	Ser	Gly	Ser	Phe	Arg	Pro	Asp	Ser	Pro	Gly
385					390					395				Ser
Gly	Asn	Ala	Arg	Pro	Asn	Asn	Pro	Asp	Trp	Gly	Thr	Phe	Glu	Glu
				405					410					415
Ser	Gly	Asn	Val	Ser	Pro	Gly	Thr	Arg	Arg	Glu	Tyr	His	Thr	Glu
			420					425					430	Lys
Leu	Val	Thr	Lys	Gly	Asp	Lys	Glu	Leu	Arg	Thr	Gly	Lys	Glu	Lys
			435				440					445		Val
Thr	Ser	Gly	Ser	Thr	Thr	Thr	Thr	Arg	Arg	Ser	Cys	Ser	Lys	Thr
							455				460			Val
Thr	Lys	Thr	Val	Ile	Gly	Pro	Asp	Gly	His	Lys	Glu	Val	Thr	Lys
465					470					475				Glu
Val	Val	Thr	Ser	Glu	Asp	Gly	Ser	Asp	Cys	Pro	Glu	Ala	Met	Asp
				485					490					495
Gly	Thr	Leu	Ser	Gly	Ile	Gly	Thr	Leu	Asp	Gly	Phe	Arg	His	Arg
			500					505					510	His
Pro	Asp	Glu	Ala	Ala	Phe	Phe	Asp	Thr	Ala	Ser	Thr	Gly	Lys	Thr
			515				520					525		Phe

63

Pro Gly Phe Phe Ser Pro Met Leu Gly Glu Phe Val Ser Glu Thr Glu
 530 535 540

Ser Arg Gly Ser Glu Ser Gly Ile Phe Thr Asn Thr Lys Glu Ser Ser
 545 550 555 560

Ser His His Pro Gly Ile Ala Glu Phe Pro Ser Arg Gly Lys Ser Ser
 565 570 575

Ser Tyr Ser Lys Gln Phe Thr Ser Ser Thr Ser Tyr Asn Arg Gly Asp
 580 585 590

Ser Thr Phe Glu Ser Lys Ser Tyr Lys Met Ala Asp Glu Ala Gly Ser
 595 600 605

Glu Ala Asp His Glu Gly Thr His Ser Thr Lys Arg Gly His Ala Lys
 610 615 620

Ser Arg Pro Val Arg Gly Ile His Thr Ser Pro Leu Gly Lys Pro Ser
 625 630 635 640

Leu Ser Pro

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 4027 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 3..4013

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

AC ATG GCA GTG AGT CAT GGG AGG GAG AGC AAG CCT CTG ACT GCT CAA	47
Met Ala Val Ser His Gly Arg Glu Ser Lys Pro Leu Thr Ala Gln	
1 5 10 15	
CAG ACA ACC AAA CTG GAT GCT CCC ACT AAC CTC CAG TTT GTC AAT GAA	95
Gln Thr Thr Lys Leu Asp Ala Pro Thr Asn Leu Gln Phe Val Asn Glu	
20 25 30	
ACT GAT TCT ACT GTC CTG GTG AGA TGG ACT CCA CCT CGG GCC CAG ATA	143
Thr Asp Ser Thr Val Leu Val Arg Trp Thr Pro Pro Arg Ala Gln Ile	
35 40 45	

ACA	GGA	TAC	CGA	CTG	ACC	GTG	GGC	CTT	ACC	CGA	AGA	GGC	CAG	CCC	AGG	191
Thr	Gly	Tyr	Arg	Leu	Thr	Val	Gly	Leu	Thr	Arg	Arg	Gly	Gln	Pro	Arg	
		50					55					60				
CAG	TAC	AAT	GTG	GGT	CCC	TCT	GTC	TCC	AAG	TAC	CCC	CTG	AGG	AAT	CTG	239
Gln	Tyr	Asn	Val	Gly	Pro	Ser	Val	Ser	Lys	Tyr	Pro	Leu	Arg	Asn	Leu	
	65					70					75					
CAG	CCT	GCA	TCT	GAG	TAC	ACC	GTA	TCC	CTC	GTG	GCC	ATA	AAG	GGC	AAC	287
Gln	Pro	Ala	Ser	Glu	Tyr	Thr	Val	Ser	Leu	Val	Ala	Ile	Lys	Gly	Asn	
	80				85					90					95	
CAA	GAG	AGC	CCC	AAA	GCC	ACT	GGA	GTC	TTT	ACC	ACA	CTG	CAG	CCT	GGG	335
Gln	Glu	Ser	Pro	Lys	Ala	Thr	Gly	Val	Phe	Thr	Thr	Leu	Gln	Pro	Gly	
				100					105					110		
AGC	TCT	ATT	CCA	CCT	TAC	AAC	ACC	GAG	GTG	ACT	GAG	ACC	ACC	ATC	GTG	383
Ser	Ser	Ile	Pro	Pro	Tyr	Asn	Thr	Glu	Val	Thr	Glu	Thr	Thr	Ile	Val	
			115						120					125		
ATC	ACA	TGG	ACG	CCT	GCT	CCA	AGA	ATT	GGT	TTT	AAG	CTG	GGT	GTA	CGA	431
Ile	Thr	Trp	Thr	Pro	Ala	Pro	Arg	Ile	Gly	Phe	Lys	Leu	Gly	Val	Arg	
		130					135					140				
CCA	AGC	CAG	GGA	GGA	GAG	GCA	CCA	CGA	GAA	GTG	ACT	TCA	GAC	TCA	GGA	479
Pro	Ser	Gln	Gly	Gly	Glu	Ala	Pro	Arg	Glu	Val	Thr	Ser	Asp	Ser	Gly	
	145					150					155					
AGC	ATC	GTT	GTG	TCC	GGC	TTG	ACT	CCA	GGA	GTA	GAA	TAC	GTC	TAC	ACC	527
Ser	Ile	Val	Val	Ser	Gly	Leu	Thr	Pro	Gly	Val	Glu	Tyr	Val	Tyr	Thr	
	160				165					170					175	
ATC	CAA	GTC	CTG	AGA	GAT	GGA	CAG	GAA	AGA	GAT	GCG	CCA	ATT	GTA	AAC	575
Ile	Gln	Val	Leu	Arg	Asp	Gly	Gln	Glu	Arg	Asp	Ala	Pro	Ile	Val	Asn	
				180					185					190		
AAA	GTG	GTG	ACA	CCA	TTG	TCT	CCA	CCA	ACA	AAC	TTG	CAT	CTG	GAG	GCA	623
Lys	Val	Val	Thr	Pro	Leu	Ser	Pro	Pro	Thr	Asn	Leu	His	Leu	Glu	Ala	
			195				200					205				
AAC	CCT	GAC	ACT	GGA	GTG	CTC	ACA	GTC	TCC	TGG	GAG	AGG	AGC	ACC	ACC	671
Asn	Pro	Asp	Thr	Gly	Val	Leu	Thr	Val	Ser	Trp	Glu	Arg	Ser	Thr	Thr	
		210					215					220				
CCA	GAC	ATT	ACT	GGT	TAT	AGA	ATT	ACC	ACA	ACC	CCT	ACA	AAC	GGC	CAG	719
Pro	Asp	Ile	Thr	Gly	Tyr	Arg	Ile	Thr	Thr	Thr	Pro	Thr	Asn	Gly	Gln	
	225					230					235					
CAG	GGA	AAT	TCT	TTG	GAA	GAA	GTG	GTC	CAT	GCT	GAT	CAG	AGC	TCC	TGC	767
Gln	Gly	Asn	Ser	Leu	Glu	Glu	Val	Val	His	Ala	Asp	Gln	Ser	Ser	Cys	
	240				245					250					255	
ACT	TTT	GAT	AAC	CTG	AGT	CCC	GGC	CTG	GAG	TAC	AAT	GTC	AGT	GTT	TAC	815
Thr	Phe	Asp	Asn	Leu	Ser	Pro	Gly	Leu	Glu	Tyr	Asn	Val	Ser	Val	Tyr	
				260					265						270	

ACT Thr	GTC Val	AAG Lys	GAT Asp 275	GAC Asp	AAG Lys	GAA Glu	AGT Ser	GTC Val 280	CCT Pro	ATC Ile	TCT Ser	GAT Asp 285	ACC Thr 285	ATC Ile	ATC Ile	863
CCA Pro	GAG Glu	GTG Val 290	CCC Pro	CAA Gln	CTC Leu	ACT Thr	GAC Asp 295	CTA Leu	AGC Ser	TTT Phe	GTT Val	GAT Asp 300	ATA Ile	ACC Thr	GAT Asp	911
TCA Ser	AGC Ser 305	ATC Ile	GGC Gly	CTG Leu	AGG Arg	TGG Trp 310	ACC Thr	CCG Pro	CTA Leu	AAC Asn	TCT Ser 315	TCC Ser	ACC Thr	ATT Ile	ATT Ile	959
GGG Gly 320	TAC Tyr	CGC Arg	ATC Ile	ACA Thr	GTA Val 325	GTT Val	GCG Ala	GCA Ala	GGA Gly	GAA Glu 330	GGT Gly	ATC Ile	CCT Pro	ATT Ile	TTT Phe 335	1007
GAA Glu	GAT Asp	TTT Phe	GTG Val	TAC Tyr 340	TCC Ser	TCA Ser	GTA Val	GGA Gly	TAC Tyr 345	TAC Tyr	ACA Thr	GTC Val	ACA Thr	GGG Gly 350	CTG Leu	1055
GAG Glu	CCG Pro	GGC Gly	ATT Ile 355	GAC Asp	TAT Tyr	GAT Asp	ATC Ile	AGC Ser 360	GTT Val	ATC Ile	ACT Thr	CTC Leu	ATT Ile 365	AAT Asn	GGC Gly	1103
GGC Gly	GAG Glu	AGT Ser 370	GCC Ala	CCT Pro	ACT Thr	ACA Thr	CTG Leu 375	ACA Thr	CAA Gln	CAA Gln	ACG Thr	GCT Ala 380	GTT Val	CCT Pro	CCT Pro	1151
CCC Pro	ACT Thr 385	GAC Asp	CTG Leu	CGA Arg	TTC Phe	ACC Thr 390	AAC Asn	ATT Ile	GGT Gly	CCA Pro	GAC Asp 395	ACC Thr	ATG Met	CGT Arg	GTC Val	1199
ACC Thr 400	TGG Trp	GCT Ala	CCA Pro	CCC Pro	CCA Pro 405	TCC Ser	ATT Ile	GAT Asp	TTA Leu	ACC Thr 410	AAC Asn	TTC Phe	CTG Leu	GTG Val 415	CGT Arg	1247
TAC Tyr	TCA Ser	CCT Pro	GTG Val	AAA Lys 420	AAT Asn	GAG Glu	GAA Glu	GAT Asp	GTT Val 425	GCA Ala	GAG Glu	TTG Leu	TCA Ser	ATT Ile 430	TCT Ser	1295
CCT Pro	TCA Ser	GAC Asp	AAT Asn 435	GCA Ala	GTG Val	GTC Val	TTA Leu 440	ACA Thr	AAT Asn	CTC Leu	CTG Leu	CCT Pro	GGT Gly 445	ACA Thr	GAA Glu	1343
TAT Tyr	GTA Val	GTG Val 450	AGT Ser	GTC Val	TCC Ser	AGT Ser	GTC Val 455	TAC Tyr	GAA Glu	CAA Gln	CAT His	GAG Glu 460	AGC Ser	ACA Thr	CCT Pro	1391
CTT Leu	AGA Arg 465	GGA Gly	AGA Arg	CAG Gln	AAA Lys	ACA Thr 470	GGT Gly	CTT Leu	GAT Asp	TCC Ser	CCA Pro 475	ACT Thr	GGC Gly	ATT Ile	GAC Asp	1439

TTT	TCT	GAT	ATT	ACT	GCC	AAC	TCT	TTT	ACT	GTG	CAC	TGG	ATT	GCT	CCT	1487
Phe	Ser	Asp	Ile	Thr	Ala	Asn	Ser	Phe	Thr	Val	His	Trp	Ile	Ala	Pro	
480					485					490					495	
CGA	GCC	ACC	ATC	ACT	GGC	TAC	AGG	ATC	CGC	CAT	CAT	CCC	GAG	CAC	TTC	1535
Arg	Ala	Thr	Ile	Thr	Gly	Tyr	Arg	Ile	Arg	His	His	Pro	Glu	His	Phe	
				500					505					510		
AGT	GGG	AGA	CCT	CGA	GAA	GAT	CGG	GTG	CCC	CAC	TCT	CGG	AAT	TCC	ATC	1583
Ser	Gly	Arg	Pro	Arg	Glu	Asp	Arg	Val	Pro	His	Ser	Arg	Asn	Ser	Ile	
			515					520					525			
ACC	CTC	ACC	AAC	CTC	ACT	CCA	GGC	ACA	GAG	TAT	GTG	GTC	AGC	ATC	GTT	1631
Thr	Leu	Thr	Asn	Leu	Thr	Pro	Gly	Thr	Glu	Tyr	Val	Val	Ser	Ile	Val	
		530					535					540				
GCT	CTT	AAT	GGC	AGA	GAG	GAA	AGT	CCC	TTA	TTG	ATT	GGC	CAA	CAA	TCA	1679
Ala	Leu	Asn	Gly	Arg	Glu	Glu	Ser	Pro	Leu	Leu	Ile	Gly	Gln	Gln	Ser	
	545					550					555					
ACA	GTT	TCT	GAT	GTT	CCG	AGG	GAC	CTG	GAA	GTT	GTT	GCT	GCG	ACC	CCC	1727
Thr	Val	Ser	Asp	Val	Pro	Arg	Asp	Leu	Glu	Val	Val	Ala	Ala	Thr	Pro	
560					565					570					575	
ACC	AGC	CTA	CTG	ATC	AGC	TGG	GAT	GCT	CCT	GCT	GTC	ACA	GTG	AGA	TAT	1775
Thr	Ser	Leu	Leu	Ile	Ser	Trp	Asp	Ala	Pro	Ala	Val	Thr	Val	Arg	Tyr	
				580					585					590		
TAC	AGG	ATC	ACT	TAC	GGA	GAA	ACA	GGA	GGA	AAT	AGC	CCT	GTC	CAG	GAG	1823
Tyr	Arg	Ile	Thr	Tyr	Gly	Glu	Thr	Gly	Gly	Asn	Ser	Pro	Val	Gln	Glu	
			595					600					605			
TTC	ACT	GTG	CCT	GGG	AGC	AAG	TCT	ACA	GCT	ACC	ATC	AGC	GGC	CTT	AAA	1871
Phe	Thr	Val	Pro	Gly	Ser	Lys	Ser	Thr	Ala	Thr	Ile	Ser	Gly	Leu	Lys	
		610					615					620				
CCT	GGA	GTT	GAT	TAT	ACC	ATC	ACT	GTG	TAT	GCT	GTC	ACT	GGC	CGT	GGA	1919
Pro	Gly	Val	Asp	Tyr	Thr	Ile	Thr	Val	Tyr	Ala	Val	Thr	Gly	Arg	Gly	
	625					630					635					
GAC	AGC	CCC	GCA	AGC	AGC	AAG	CCA	ATT	TCC	ATT	AAT	TAC	CGA	ACA	GAA	1967
Asp	Ser	Pro	Ala	Ser	Ser	Lys	Pro	Ile	Ser	Ile	Asn	Tyr	Arg	Thr	Glu	
640					645					650					655	
ATT	GAC	AAA	CCA	TCC	CAG	ATG	CAA	GTG	ACC	GAT	GTT	CAG	GAC	AAC	AGC	2015
Ile	Asp	Lys	Pro	Ser	Gln	Met	Gln	Val	Thr	Asp	Val	Gln	Asp	Asn	Ser	
				660					665					670		
ATT	AGT	GTC	AAG	TGG	CTG	CCT	TCA	AGT	TCC	CCT	GTT	ACT	GGT	TAC	AGA	2063
Ile	Ser	Val	Lys	Trp	Leu	Pro	Ser	Ser	Ser	Pro	Val	Thr	Gly	Tyr	Arg	
			675					680					685			
GTA	ACC	ACC	ACT	CCC	AAA	AAT	GGA	CCA	GGA	CCA	ACA	AAA	ACT	AAA	ACT	2111
Val	Thr	Thr	Thr	Pro	Lys	Asn	Gly	Pro	Gly	Pro	Thr	Lys	Thr	Lys	Thr	
			690				695						700			

GCA Ala 705	GGT Gly	CCA Pro	GAT Asp	CAA Gln	ACA Thr	GAA Glu 710	ATG Met	ACT Thr	ATT Ile	GAA Glu 715	GGC Gly	TTG Leu	CAG Gln	CCC Pro	ACA Thr	2159
GTG Val 720	GAG Glu	TAT Tyr	GTG Val	GTT Val	AGT Ser 725	GTC Val	TAT Tyr	GCT Ala	CAG Gln	AAT Asn 730	CCA Pro	AGC Ser	GGA Gly	GAG Glu	AGT Ser 735	2207
CAG Gln	CCT Pro	CTG Leu	GTT Val	CAG Gln 740	ACT Thr	GCA Ala	GTA Val	ACC Thr	AAC Asn 745	ATT Ile	GAT Asp	CGC Arg	CCT Pro	AAA Lys 750	GGA Gly	2255
CTG Leu	GCA Ala	TTC Phe	ACT Thr 755	GAT Asp	GTG Val	GAT Asp	GTC Val	GAT Asp 760	TCC Ser	ATC Ile	AAA Lys	ATT Ile	GCT Ala 765	TGG Trp	GAA Glu	2303
AGC Ser	CCA Pro	CAG Gln 770	GGG Gly	CAA Gln	GTT Val	TCC Ser	AGG Arg 775	TAC Tyr	AGG Arg	GTG Val	ACC Thr	TAC Tyr 780	TCG Ser	AGC Ser	CCT Pro	2351
GAG Glu 785	GAT Asp	GGA Gly	ATC Ile	CAT His	GAG Glu	CTA Leu 790	TTC Phe	CCT Pro	GCA Ala	CCT Pro	GAT Asp 795	GGT Gly	GAA Glu	GAA Glu	GAC Asp	2399
ACT Thr 800	GCA Ala	GAG Glu	CTG Leu	CAA Gln	GGC Gly 805	CTC Leu	AGA Arg	CCG Pro	GGT Gly	TCT Ser 810	GAG Glu	TAC Tyr	ACA Thr	GTC Val	AGT Ser 815	2447
GTG Val	GTT Val	GCC Ala	TTG Leu	CAC His 820	GAT Asp	GAT Asp	ATG Met	GAG Glu	AGC Ser 825	CAG Gln	CCC Pro	CTG Leu	ATT Ile	GGA Gly	ACC Thr 830	2495
CAG Gln	TCC Ser	ACA Thr	GCT Ala 835	ATT Ile	CCT Pro	GCA Ala	CCA Pro	ACT Thr 840	GAC Asp	CTG Leu	AAG Lys	TTC Phe	ACT Thr 845	CAG Gln	GTC Val	2543
ACA Thr	CCC Pro	ACA Thr 850	AGC Ser	CTG Leu	AGC Ser	GCC Ala	CAG Gln 855	TGG Trp	ACA Thr	CCA Pro	CCC Pro	AAT Asn 860	GTT Val	CAG Gln	CTC Leu	2591
ACT Thr 865	GGA Gly	TAT Tyr	CGA Arg	GTG Val	CGG Arg	GTG Val 870	ACC Thr	CCC Pro	AAG Lys	GAG Glu	AAG Lys 875	ACC Thr	GGA Gly	CCA Pro	ATG Met	2639
AAA Lys 880	GAA Glu	ATC Ile	AAC Asn	CTT Leu	GCT Ala 885	CCT Pro	GAC Asp	AGC Ser	TCA Ser	TCC Ser 890	GTG Val	GTT Val	GTA Val	TCA Ser	GGA Gly 895	2687
CTT Leu	ATG Met	GTG Val	GCC Ala	ACC Thr 900	AAA Lys	TAT Tyr	GAA Glu	GTG Val	AGT Ser 905	GTC Val	TAT Tyr	GCT Ala	CTT Leu	AAG Lys	GAC Asp 910	2735

ACT	TTG	ACA	AGC	AGA	CCA	GCT	CAG	GGT	GTT	GTC	ACC	ACT	CTG	GAG	GGA	2783
Thr	Leu	Thr	Ser	Arg	Pro	Ala	Gln	Gly	Val	Val	Thr	Thr	Leu	Glu	Gly	
			915					920					925			
GGA	AAT	TTT	AAG	AGC	CAG	CTT	CAG	AAG	GTA	CCC	CCA	GAG	TGG	AAG	GCA	2831
Gly	Asn	Phe	Lys	Ser	Gln	Leu	Gln	Lys	Val	Pro	Pro	Glu	Trp	Lys	Ala	
		930					935					940				
TTA	ACA	GAC	ATG	CCG	CAG	ATG	AGA	ATG	GAG	TTA	GAG	AGA	CCT	GGT	GGA	2879
Leu	Thr	Asp	Met	Pro	Gln	Met	Arg	Met	Glu	Leu	Glu	Arg	Pro	Gly	Gly	
	945					950					955					
AAT	GAG	ATT	ACT	CGA	GGA	GGC	TCC	ACC	TCT	TAT	GGA	ACC	GGA	TCA	GAG	2927
Asn	Glu	Ile	Thr	Arg	Gly	Gly	Ser	Thr	Ser	Tyr	Gly	Thr	Gly	Ser	Glu	
960					965					970					975	
ACG	GAA	AGC	CCC	AGG	AAC	CCT	AGC	AGT	GCT	GGA	AGC	TGG	AAC	TCT	GGG	2975
Thr	Glu	Ser	Pro	Arg	Asn	Pro	Ser	Ser	Ala	Gly	Ser	Trp	Asn	Ser	Gly	
				980					985					990		
AGC	TCT	GGA	CCT	GGA	AGT	ACT	GGA	AAC	CGA	AAC	CCT	GGG	AGC	TCT	GGG	3023
Ser	Ser	Gly	Pro	Gly	Ser	Thr	Gly	Asn	Arg	Asn	Pro	Gly	Ser	Ser	Gly	
			995					1000					1005			
ACT	GGA	GGG	ACT	GCA	ACC	TGG	AAA	CCT	GGG	AGC	TCT	GGA	CCT	GGA	AGT	3071
Thr	Gly	Gly	Thr	Ala	Thr	Trp	Lys	Pro	Gly	Ser	Ser	Gly	Pro	Gly	Ser	
		1010					1015					1020				
GCT	GGA	AGC	TGG	AAC	TCT	GGG	AGC	TCT	GGA	ACT	GGA	AGT	ACT	GGA	AAC	3119
Ala	Gly	Ser	Trp	Asn	Ser	Gly	Ser	Ser	Gly	Thr	Gly	Ser	Thr	Gly	Asn	
	1025					1030					1035					
CAA	AAC	CCT	GGG	AGC	CCT	AGA	CCT	GGT	AGT	ACC	GGA	ACC	TGG	AAT	CCT	3167
Gln	Asn	Pro	Gly	Ser	Pro	Arg	Pro	Gly	Ser	Thr	Gly	Thr	Trp	Asn	Pro	
1040					1045					1050					1055	
GGC	AGC	TCT	GAA	CGC	GGA	AGT	GCT	GGG	CAC	TGG	ACC	TCT	GAG	AGC	TCT	3215
Gly	Ser	Ser	Glu	Arg	Gly	Ser	Ala	Gly	His	Trp	Thr	Ser	Glu	Ser	Ser	
				1060				1065						1070		
GTA	TCT	GGT	AGT	ACT	GGA	CAA	TGG	CAC	TCT	GAA	TCT	GGA	AGT	TTT	AGG	3263
Val	Ser	Gly	Ser	Thr	Gly	Gln	Trp	His	Ser	Glu	Ser	Gly	Ser	Phe	Arg	
			1075					1080					1085			
CCA	GAT	AGC	CCA	GGC	TCT	GGG	AAC	GCG	AGG	CCT	AAC	AAC	CCA	GAC	TGG	3311
Pro	Asp	Ser	Pro	Gly	Ser	Gly	Asn	Ala	Arg	Pro	Asn	Asn	Pro	Asp	Trp	
		1090					1095					1100				
GGC	ACA	TTT	GAA	GAG	GTG	TCA	GGA	AAT	GTA	AGT	CCA	GGG	ACA	AGG	AGA	3359
Gly	Thr	Phe	Glu	Glu	Val	Ser	Gly	Asn	Val	Ser	Pro	Gly	Thr	Arg	Arg	
	1105					1110					1115					
GAG	TAC	CAC	ACA	GAA	AAA	CTG	GTC	ACT	AAA	GGA	GAT	AAA	GAG	CTC	AGG	3407
Glu	Tyr	His	Thr	Glu	Lys	Leu	Val	Thr	Lys	Gly	Asp	Lys	Glu	Leu	Arg	
1120					1125					1130					1135	

ACT GGT AAA GAG AAG GTC ACC TCT GGT AGC ACA ACC ACC ACG CGT CGT	3455
Thr Gly Lys Glu Lys Val Thr Ser Gly Ser Thr Thr Thr Thr Arg Arg	
1140 1145 1150	
TCA TGC TCT AAA ACC GTT ACT AAG ACT GTT ATT GGT CCT GAT GGT CAC	3503
Ser Cys Ser Lys Thr Val Thr Lys Thr Val Ile Gly Pro Asp Gly His	
1155 1160 1165	
AAA GAA GTT ACC AAA GAA GTG GTG ACC TCC GAA GAT GGT TCT GAC TGT	3551
Lys Glu Val Thr Lys Glu Val Val Thr Ser Glu Asp Gly Ser Asp Cys	
1170 1175 1180	
CCC GAG GCA ATG GAT TTA GGC ACA TTG TCT GGC ATA GGT ACT CTG GAT	3599
Pro Glu Ala Met Asp Leu Gly Thr Leu Ser Gly Ile Gly Thr Leu Asp	
1185 1190 1195	
GGG TTC CGC CAT AGG CAC CCT GAT GAA GCT GCC TTC TTC GAC ACT GCC	3647
Gly Phe Arg His Arg His Pro Asp Glu Ala Ala Phe Phe Asp Thr Ala	
1200 1205 1210 1215	
TCA ACT GGA AAA ACA TTC CCA GGT TTC TTC TCA CCT ATG TTA GGA GAG	3695
Ser Thr Gly Lys Thr Phe Pro Gly Phe Phe Ser Pro Met Leu Gly Glu	
1220 1225 1230	
TTT GTC AGT GAG ACT GAG TCT AGG GGC TCA GAA TCT GGC ATC TTC ACA	3743
Phe Val Ser Glu Thr Glu Ser Arg Gly Ser Glu Ser Gly Ile Phe Thr	
1235 1240 1245	
AAT ACA AAG GAA TCC AGT TCT CAT CAC CCT GGG ATA GCT GAA TTC CCT	3791
Asn Thr Lys Glu Ser Ser Ser His His Pro Gly Ile Ala Glu Phe Pro	
1250 1255 1260	
TCC CGT GGT AAA TCT TCA AGT TAC AGC AAA CAA TTT ACT AGT AGC ACG	3839
Ser Arg Gly Lys Ser Ser Ser Tyr Ser Lys Lys Phe Thr Ser Ser Thr	
1265 1270 1275	
AGT TAC AAC AGA GGA GAC TCC ACA TTT GAA AGC AAG AGC TAT AAA ATG	3887
Ser Tyr Asn Arg Gly Asp Ser Thr Phe Glu Ser Lys Ser Tyr Lys Met	
1280 1285 1290 1295	
GCA GAT GAG GCC GGA AGT GAA GCC GAT CAT GAA GGA ACA CAT AGC ACC	3935
Ala Asp Glu Ala Gly Ser Glu Ala Asp His Glu Gly Thr His Ser Thr	
1300 1305 1310	
AAG AGA GGC CAT GCT AAA TCT CGC CCT GTC AGA GGT ATC CAC ACT TCT	3983
Lys Arg Gly His Ala Lys Ser Arg Pro Val Arg Gly Ile His Thr Ser	
1315 1320 1325	
CCT TTG GGG AAG CCT TCC CTG TCC CCC TAGACTAAGT TAAATAT	4027
Pro Leu Gly Lys Pro Ser Leu Ser Pro	
1330 1335	

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1336 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```

Met Ala Val Ser His Gly Arg Glu Ser Lys Pro Leu Thr Ala Gln Gln
 1           5           10           15
Thr Thr Lys Leu Asp Ala Pro Thr Asn Leu Gln Phe Val Asn Glu Thr
          20           25           30
Asp Ser Thr Val Leu Val Arg Trp Thr Pro Pro Arg Ala Gln Ile Thr
          35           40           45
Gly Tyr Arg Leu Thr Val Gly Leu Thr Arg Arg Gly Gln Pro Arg Gln
          50           55           60
Tyr Asn Val Gly Pro Ser Val Ser Lys Tyr Pro Leu Arg Asn Leu Gln
          65           70           75           80
Pro Ala Ser Glu Tyr Thr Val Ser Leu Val Ala Ile Lys Gly Asn Gln
          85           90           95
Glu Ser Pro Lys Ala Thr Gly Val Phe Thr Thr Leu Gln Pro Gly Ser
          100          105          110
Ser Ile Pro Pro Tyr Asn Thr Glu Val Thr Glu Thr Thr Ile Val Ile
          115          120          125
Thr Trp Thr Pro Ala Pro Arg Ile Gly Phe Lys Leu Gly Val Arg Pro
          130          135          140
Ser Gln Gly Gly Glu Ala Pro Arg Glu Val Thr Ser Asp Ser Gly Ser
          145          150          155          160
Ile Val Val Ser Gly Leu Thr Pro Gly Val Glu Tyr Val Tyr Thr Ile
          165          170          175
Gln Val Leu Arg Asp Gly Gln Glu Arg Asp Ala Pro Ile Val Asn Lys
          180          185          190
Val Val Thr Pro Leu Ser Pro Pro Thr Asn Leu His Leu Glu Ala Asn
          195          200          205
Pro Asp Thr Gly Val Leu Thr Val Ser Trp Glu Arg Ser Thr Thr Pro
          210          215          220
Asp Ile Thr Gly Tyr Arg Ile Thr Thr Thr Pro Thr Asn Gly Gln Gln
          225          230          235          240

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Gly Asn Ser Leu Glu Glu Val Val His Ala Asp Gln Ser Ser Cys Thr
 245 250 255
 Phe Asp Asn Leu Ser Pro Gly Leu Glu Tyr Asn Val Ser Val Tyr Thr
 260 265 270
 Val Lys Asp Asp Lys Glu Ser Val Pro Ile Ser Asp Thr Ile Ile Pro
 275 280 285
 Glu Val Pro Gln Leu Thr Asp Leu Ser Phe Val Asp Ile Thr Asp Ser
 290 295 300
 Ser Ile Gly Leu Arg Trp Thr Pro Leu Asn Ser Ser Thr Ile Ile Gly
 305 310 315 320
 Tyr Arg Ile Thr Val Val Ala Ala Gly Glu Gly Ile Pro Ile Phe Glu
 325 330 335
 Asp Phe Val Tyr Ser Ser Val Gly Tyr Tyr Thr Val Thr Gly Leu Glu
 340 345 350
 Pro Gly Ile Asp Tyr Asp Ile Ser Val Ile Thr Leu Ile Asn Gly Gly
 355 360 365
 Glu Ser Ala Pro Thr Thr Leu Thr Gln Gln Thr Ala Val Pro Pro Pro
 370 375 380
 Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met Arg Val Thr
 385 390 395 400
 Trp Ala Pro Pro Pro Ser Ile Asp Leu Thr Asn Phe Leu Val Arg Tyr
 405 410 415
 Ser Pro Val Lys Asn Glu Glu Asp Val Ala Glu Leu Ser Ile Ser Pro
 420 425 430
 Ser Asp Asn Ala Val Val Leu Thr Asn Leu Leu Pro Gly Thr Glu Tyr
 435 440 445
 Val Val Ser Val Ser Ser Val Tyr Glu Gln His Glu Ser Thr Pro Leu
 450 455 460
 Arg Gly Arg Gln Lys Thr Gly Leu Asp Ser Pro Thr Gly Ile Asp Phe
 465 470 475 480
 Ser Asp Ile Thr Ala Asn Ser Phe Thr Val His Trp Ile Ala Pro Arg
 485 490 495
 Ala Thr Ile Thr Gly Tyr Arg Ile Arg His His Pro Glu His Phe Ser
 500 505 510
 Gly Arg Pro Arg Glu Asp Arg Val Pro His Ser Arg Asn Ser Ile Thr
 515 520 525

Leu Thr Asn Leu Thr Pro Gly Thr Glu Tyr Val Val Ser Ile Val Ala
 530 535 540
 Leu Asn Gly Arg Glu Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser Thr
 545 550 555 560
 Val Ser Asp Val Pro Arg Asp Leu Glu Val Val Ala Ala Thr Pro Thr
 565 570 575
 Ser Leu Leu Ile Ser Trp Asp Ala Pro Ala Val Thr Val Arg Tyr Tyr
 580 585 590
 Arg Ile Thr Tyr Gly Glu Thr Gly Gly Asn Ser Pro Val Gln Glu Phe
 595 600 605
 Thr Val Pro Gly Ser Lys Ser Thr Ala Thr Ile Ser Gly Leu Lys Pro
 610 615 620
 Gly Val Asp Tyr Thr Ile Thr Val Tyr Ala Val Thr Gly Arg Gly Asp
 625 630 635 640
 Ser Pro Ala Ser Ser Lys Pro Ile Ser Ile Asn Tyr Arg Thr Glu Ile
 645 650 655
 Asp Lys Pro Ser Gln Met Gln Val Thr Asp Val Gln Asp Asn Ser Ile
 660 665 670
 Ser Val Lys Trp Leu Pro Ser Ser Ser Pro Val Thr Gly Tyr Arg Val
 675 680 685
 Thr Thr Thr Pro Lys Asn Gly Pro Gly Pro Thr Lys Thr Lys Thr Ala
 690 695 700
 Gly Pro Asp Gln Thr Glu Met Thr Ile Glu Gly Leu Gln Pro Thr Val
 705 710 715 720
 Glu Tyr Val Val Ser Val Tyr Ala Gln Asn Pro Ser Gly Glu Ser Gln
 725 730 735
 Pro Leu Val Gln Thr Ala Val Thr Asn Ile Asp Arg Pro Lys Gly Leu
 740 745 750
 Ala Phe Thr Asp Val Asp Val Asp Ser Ile Lys Ile Ala Trp Glu Ser
 755 760 765
 Pro Gln Gly Gln Val Ser Arg Tyr Arg Val Thr Tyr Ser Ser Pro Glu
 770 775 780
 Asp Gly Ile His Glu Leu Phe Pro Ala Pro Asp Gly Glu Glu Asp Thr
 785 790 795 800
 Ala Glu Leu Gln Gly Leu Arg Pro Gly Ser Glu Tyr Thr Val Ser Val
 805 810 815

Val Ala Leu His Asp Asp Met Glu Ser Gln Pro Leu Ile Gly Thr Gln
 820 825 830
 Ser Thr Ala Ile Pro Ala Pro Thr Asp Leu Lys Phe Thr Gln Val Thr
 835 840 845
 Pro Thr Ser Leu Ser Ala Gln Trp Thr Pro Pro Asn Val Gln Leu Thr
 850 855 860
 Gly Tyr Arg Val Arg Val Thr Pro Lys Glu Lys Thr Gly Pro Met Lys
 865 870 875 880
 Glu Ile Asn Leu Ala Pro Asp Ser Ser Ser Val Val Val Ser Gly Leu
 885 890 895
 Met Val Ala Thr Lys Tyr Glu Val Ser Val Tyr Ala Leu Lys Asp Thr
 900 905 910
 Leu Thr Ser Arg Pro Ala Gln Gly Val Val Thr Thr Leu Glu Gly Gly
 915 920 925
 Asn Phe Lys Ser Gln Leu Gln Lys Val Pro Pro Glu Trp Lys Ala Leu
 930 935 940
 Thr Asp Met Pro Gln Met Arg Met Glu Leu Glu Arg Pro Gly Gly Asn
 945 950 955 960
 Glu Ile Thr Arg Gly Gly Ser Thr Ser Tyr Gly Thr Gly Ser Glu Thr
 965 970 975
 Glu Ser Pro Arg Asn Pro Ser Ser Ala Gly Ser Trp Asn Ser Gly Ser
 980 985 990
 Ser Gly Pro Gly Ser Thr Gly Asn Arg Asn Pro Gly Ser Ser Gly Thr
 995 1000 1005
 Gly Gly Thr Ala Thr Trp Lys Pro Gly Ser Ser Gly Pro Gly Ser Ala
 1010 1015 1020
 Gly Ser Trp Asn Ser Gly Ser Ser Gly Thr Gly Ser Thr Gly Asn Gln
 1025 1030 1035 1040
 Asn Pro Gly Ser Pro Arg Pro Gly Ser Thr Gly Thr Trp Asn Pro Gly
 1045 1050 1055
 Ser Ser Glu Arg Gly Ser Ala Gly His Trp Thr Ser Glu Ser Ser Val
 1060 1065 1070
 Ser Gly Ser Thr Gly Gln Trp His Ser Glu Ser Gly Ser Phe Arg Pro
 1075 1080 1085
 Asp Ser Pro Gly Ser Gly Asn Ala Arg Pro Asn Asn Pro Asp Trp Gly
 1090 1095 1100

Thr Phe Glu Glu Val Ser Gly Asn Val Ser Pro Gly Thr Arg Arg Glu
 1105 1110 1115 1120
 Tyr His Thr Glu Lys Leu Val Thr Lys Gly Asp Lys Glu Leu Arg Thr
 1125 1130 1135
 Gly Lys Glu Lys Val Thr Ser Gly Ser Thr Thr Thr Thr Arg Arg Ser
 1140 1145 1150
 Cys Ser Lys Thr Val Thr Lys Thr Val Ile Gly Pro Asp Gly His Lys
 1155 1160 1165
 Glu Val Thr Lys Glu Val Val Thr Ser Glu Asp Gly Ser Asp Cys Pro
 1170 1175 1180
 Glu Ala Met Asp Leu Gly Thr Leu Ser Gly Ile Gly Thr Leu Asp Gly
 1185 1190 1195 1200
 Phe Arg His Arg His Pro Asp Glu Ala Ala Phe Phe Asp Thr Ala Ser
 1205 1210 1215
 Thr Gly Lys Thr Phe Pro Gly Phe Phe Ser Pro Met Leu Gly Glu Phe
 1220 1225 1230
 Val Ser Glu Thr Glu Ser Arg Gly Ser Glu Ser Gly Ile Phe Thr Asn
 1235 1240 1245
 Thr Lys Glu Ser Ser Ser His His Pro Gly Ile Ala Glu Phe Pro Ser
 1250 1255 1260
 Arg Gly Lys Ser Ser Ser Tyr Ser Lys Gln Phe Thr Ser Ser Thr Ser
 1265 1270 1275 1280
 Tyr Asn Arg Gly Asp Ser Thr Phe Glu Ser Lys Ser Tyr Lys Met Ala
 1285 1290 1295
 Asp Glu Ala Gly Ser Glu Ala Asp His Glu Gly Thr His Ser Thr Lys
 1300 1305 1310
 Arg Gly His Ala Lys Ser Arg Pro Val Arg Gly Ile His Thr Ser Pro
 1315 1320 1325
 Leu Gly Lys Pro Ser Leu Ser Pro
 1330 1335

75

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 27 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

- (vii) IMMEDIATE SOURCE:
 (B) CLONE: ZC1551

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GATCCCCGGG GAGCTCCTCG AGGCATG

27

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 19 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

- (vii) IMMEDIATE SOURCE:
 (B) CLONE: ZC1552

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CCTCGAGGAG CTCCCCGGG

19

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 20 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

- (vii) IMMEDIATE SOURCE:
 (B) CLONE: ZC2052

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

AATTCACCAT GGCAGTGAGT

20

76

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

- (vii) IMMEDIATE SOURCE:
(B) CLONE: ZC2053

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CATGACTCAC TGCCATGGTG

20

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

- (vii) IMMEDIATE SOURCE:
(B) CLONE: ZC2491

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CTAGATTAGA ATGGGGCC

18

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

- (vii) IMMEDIATE SOURCE:
(B) CLONE: ZC2493

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CCATTCTAAT

10

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 88 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:

- (B) CLONE: ZC3521

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TCGACTTAAG GACACTTTGA CAAGCAGACC AGCTCAGGGT GTTGTACCA CTCTGGAGGG 60

AGGAAATTTT AAGAGCCAGC TTCAGAAG 88

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 88 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:

- (B) CLONE: ZC3522

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GTACCTTCTG AAGCTGGCTC TAAAATTTC CTCCTCCAG AGTGGTGACA ACACCCTGAG 60

CTGGTCTGCT TGTCAAAGTG TCCTTAAG 88

I Claim:

1. A hybrid protein comprising a tissue-binding domain from a first protein covalently linked to a cross-linking domain from a second protein.
2. A hybrid protein according to claim 1 wherein the tissue-binding domain of the first protein is a heparin binding domain of thrombospondin, a heparin binding domain of fibronectin, a collagen binding domain of fibronectin or a cell binding domain of fibronectin.
3. A hybrid protein according to claim 1 wherein the tissue-binding domain of the first protein comprises the amino acid sequence of Sequence ID No. 6 from Alanine, amino acid 2 to Glutamic acid, amino acid number 926.
4. A hybrid protein according to claim 1 wherein the cross-linking domain of the second protein comprises the carboxy-terminal 103 amino acids of loricrin; the ten amino acid repeat beginning with glutamine, amino acid number 496 of involucrin; or the 400 amino-terminal amino acids of the fibrinogen α chain.
5. A hybrid protein according to claim 1 wherein the cross-linking domain of the second protein comprises the amino acid sequence of Sequence ID No. 6 from Glycine, amino acid number 928 to Proline, amino acid number 1336.
6. A hybrid protein according to claim 1 comprising the amino acid sequence of Sequence ID Number 6 from alanine, amino acid number 2 to Proline, amino acid number 1336.
7. An isolated DNA molecule encoding a hybrid protein comprising a first DNA segment encoding a tissue-binding domain from a first protein joined to a second DNA segment encoding a cross-linking domain from a second protein.

8. A DNA molecule according to claim 7 wherein the first DNA segment encodes a heparin binding domain of thrombospondin, a heparin binding domain of fibronectin, a collagen binding domain of fibronectin, a collagen binding domain of fibronectin or a cell binding domain of fibronectin.

9. A DNA molecule according to claim 7 wherein the first DNA segment comprises the nucleotide sequence of Sequence ID No. 5 from nucleotide 3 to nucleotide 2780.

10. A DNA molecule according to claim 7 wherein the first DNA segment encodes the amino acid sequence of Sequence ID No. 6 from methionine, amino acid number 1 to glutamic acid, amino acid number 926.

11. A DNA molecule according to claim 7 wherein the second DNA segment encodes the carboxy-terminal 103 amino acids of loricrin; the ten amino acid repeat beginning with glutamine, amino acid number 496 of involucrin; or the 400 amino-terminal amino acids of the fibrinogen α chain.

12. A DNA molecule according to claim 7 wherein the second DNA segment comprises the nucleotide sequence of Sequence ID No. 5 from nucleotide 2784 to nucleotide 4013.

13. A DNA molecule according to claim 7 wherein the second DNA segment encodes the amino acid sequence of Sequence ID No. 6 from glycine, amino acid number 928 to proline, amino acid number 1336.

14. A DNA molecule according to claim 7 wherein the DNA molecule encodes the amino acid sequence of Sequence ID Number 6 from Methionine, amino acid number 1 to Proline, amino acid number 1336.

15. A DNA molecule according to claim 7 wherein the DNA molecule comprises the nucleotide sequence of Sequence ID Number 5 from nucleotide 3 to nucleotide 4013.

16. A DNA construct comprising a DNA molecule encoding a hybrid protein, wherein said DNA molecule comprises a first DNA segment encoding a tissue-binding domain from a first protein joined to a second DNA segment encoding a cross-linking domain from a second protein, and wherein said DNA molecule is operably linked to other DNA segments required for the expression of the DNA molecule.

17. A DNA construct according to claim 16 wherein the first DNA segment encodes a heparin binding domain of thrombospondin, a heparin binding domain of fibronectin, a collagen binding domain of fibronectin or a cell binding domain of fibronectin.

18. A DNA construct according to claim 16 wherein the first DNA segment comprises the nucleotide sequence of Sequence ID No. 5 from nucleotide 3 to nucleotide 2780.

19. A DNA construct according to claim 16 wherein the first DNA segment encodes the amino acid sequence of Sequence ID No. 6 from methionine, amino acid 1 to Glutamic acid, amino acid number 926.

20. A DNA construct according to claim 16 wherein the second DNA segment encodes the carboxy-terminal 103 amino acids of loricrin; the ten amino acid repeat beginning with glutamine, amino acid number 496 of involucrin; or the 400 amino-terminal amino acids of the fibrinogen α chain.

21. A DNA construct according to claim 16 wherein the second DNA segment comprises the nucleotide sequence of Sequence ID No. 5 from nucleotide 2784 to nucleotide 4013.

22. A DNA construct according to claim 16 wherein the second DNA segment encodes the amino acid sequence of Sequence ID No. 6 from glycine, amino acid number 928 to proline, amino acid number 1336.

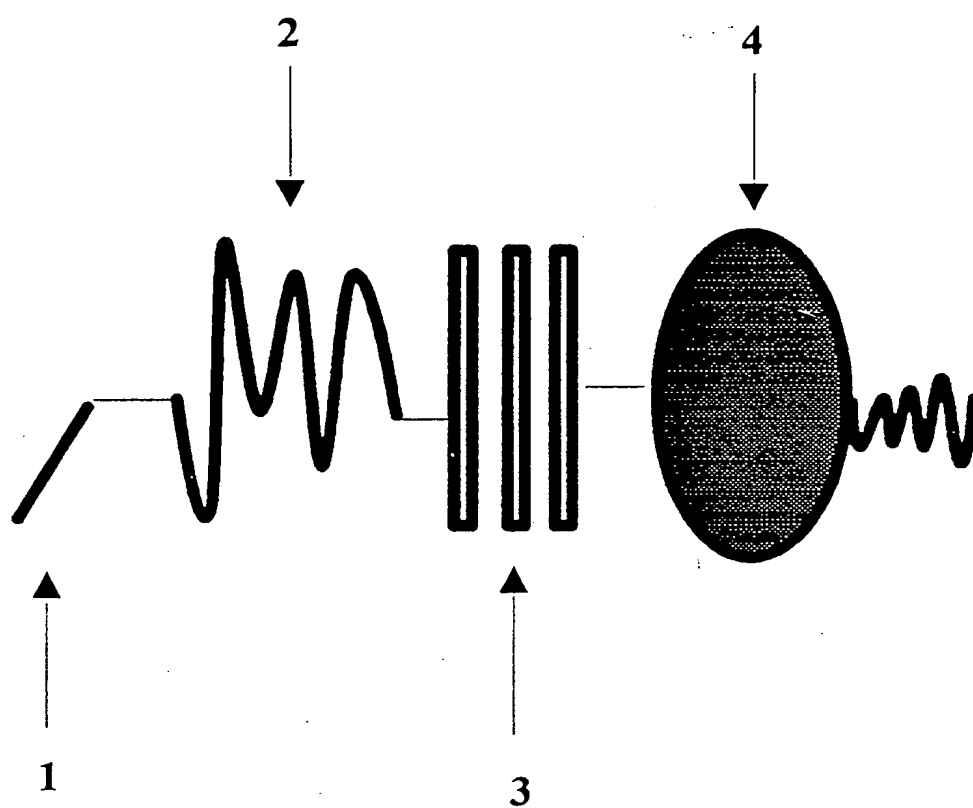
23. A DNA construct according to claim 16 wherein the DNA molecule comprises the nucleotide sequence of Sequence ID Number 5 from nucleotide 1 to nucleotide 4013.

24. A DNA construct according to claim 16 wherein the DNA molecule encodes the amino acid sequence of Sequence ID Number 6 from Methionine, amino acid number 1 to Proline, amino acid number 1336.

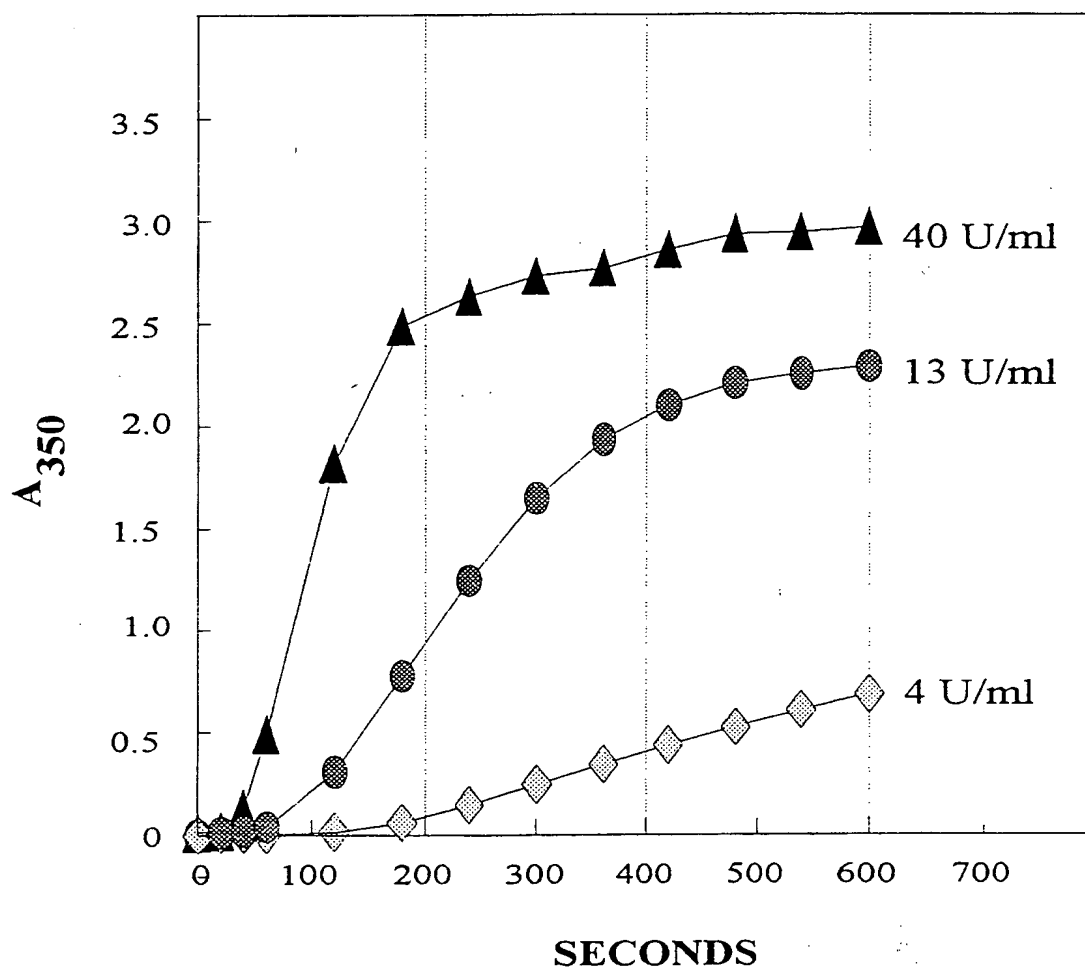
25. A host cell containing a DNA construct according to claim 16.

26. A method for producing a hybrid protein comprising culturing a host cell according to claim 25 under conditions promoting the expression of the first DNA segment.

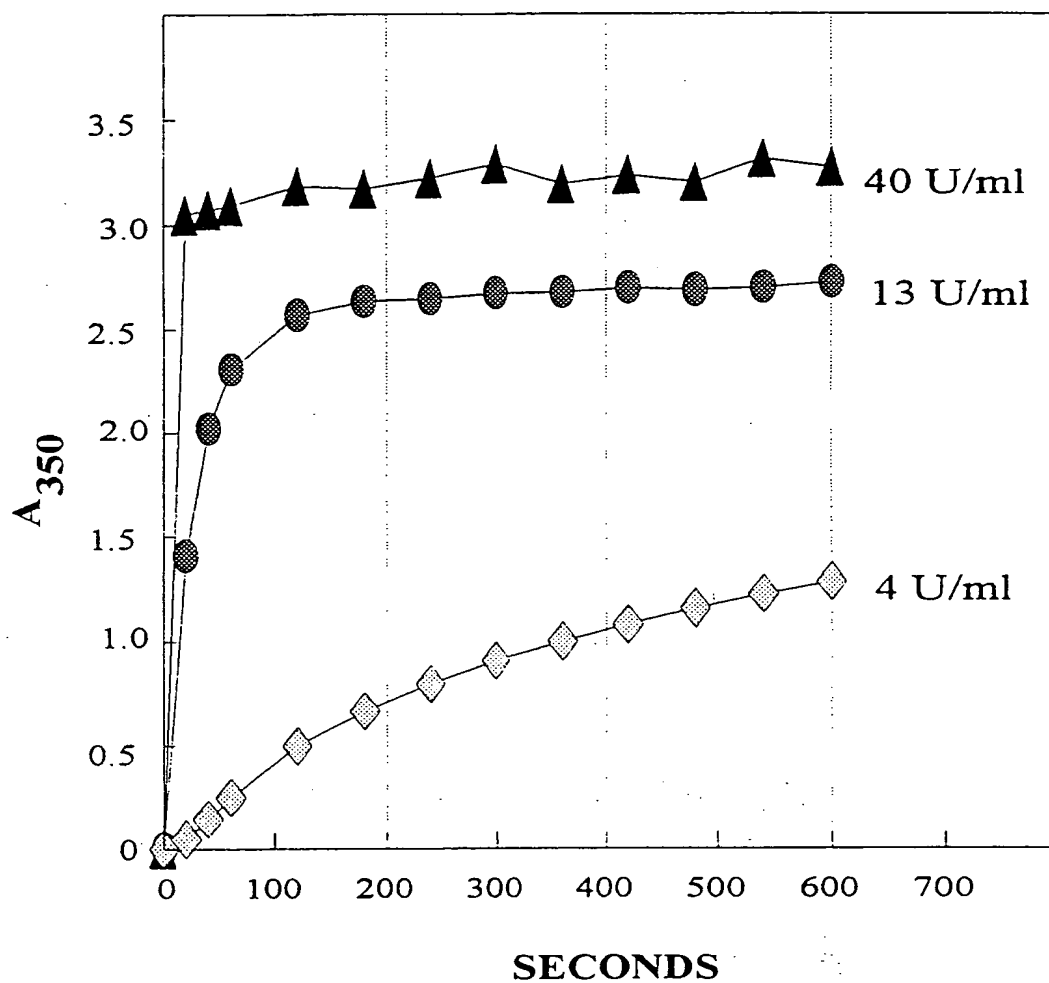
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**FIGURE 1**

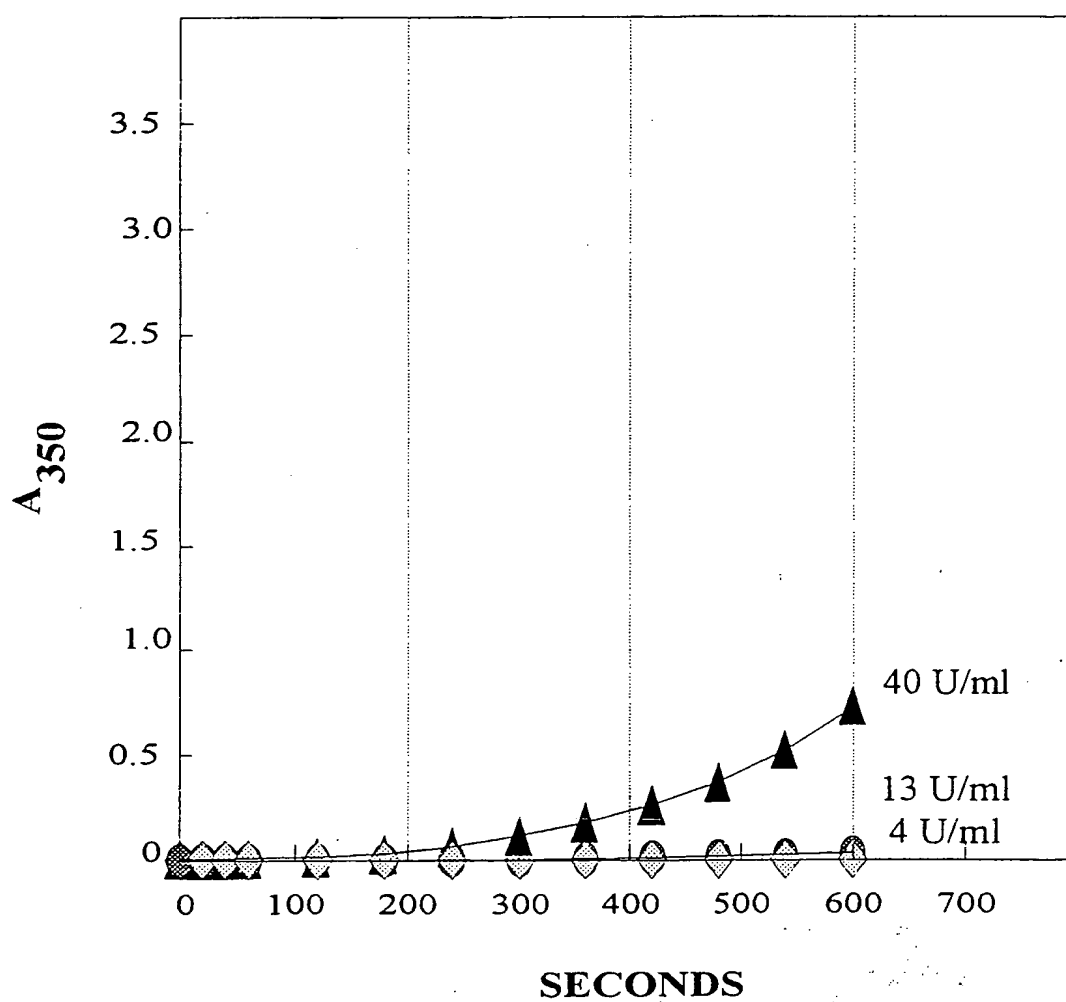
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Hybrid+FXIII+Thrombin**FIGURE 2**

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Hybrid+FXIIIa**FIGURE 3**

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FXIII+Thrombin**FIGURE 4**

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FXIIIa

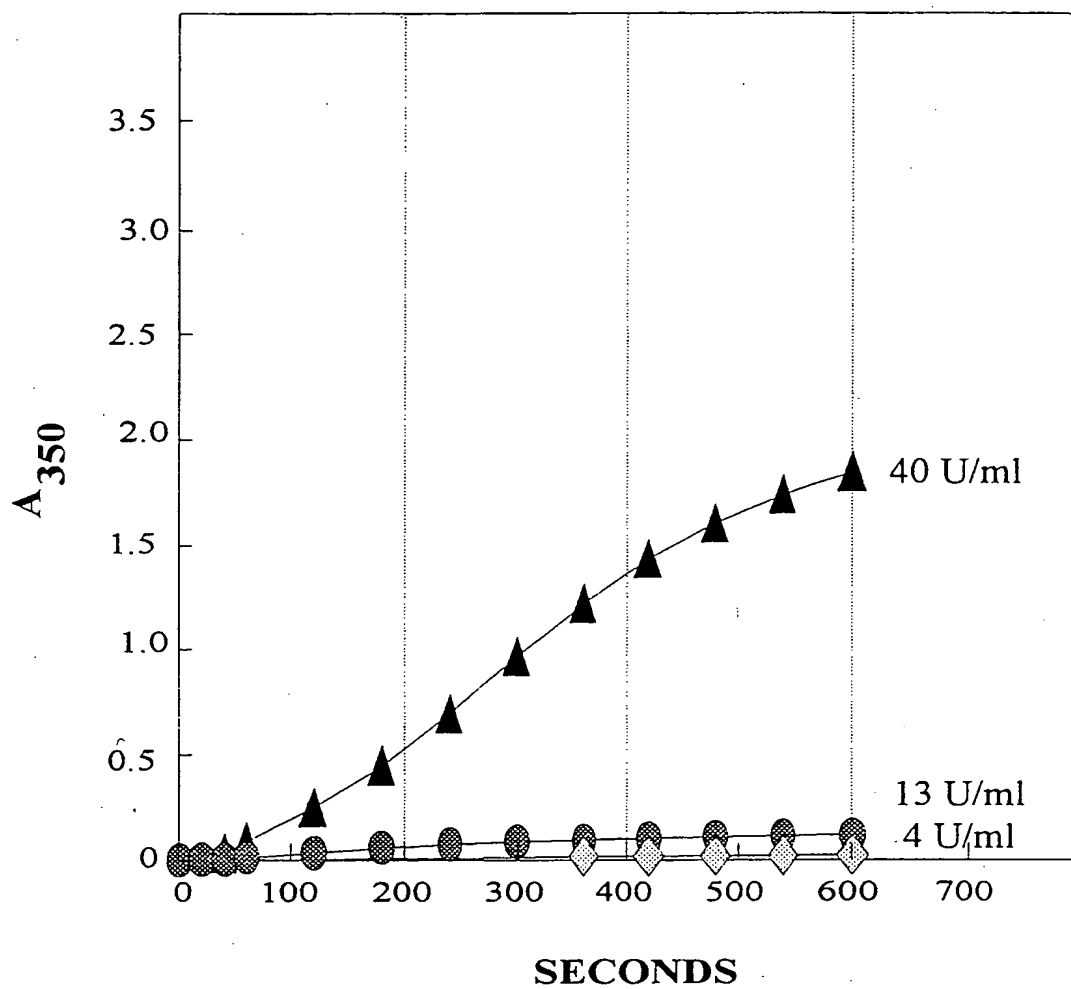


FIGURE 5